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MISCELLANEOUS PUBLICATION 3
IMMUNOFLUORESCENCE,
AN ANNOTATED BIBLIOGRAPHY
I. BACTERIAL STUDIES

Warren R. Sanborn

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DECEMBER 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

MISCELLANEOUS PUBLICATION 3

IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

I. BACTERIAL STUDIES

Warren R. Sanborn

December 1965

Pathology Division
DIRECTORATE OF MEDICAL RESEARCH

FOREWORD

The use of immunofluorescence, or fluorescent antibodies, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The expanding volume of literature covering uses of immunofluorescence bears witness to the value of the technique. Through 1954 only about 40 articles had been published. In the next two years 58 articles were published. In 1957 and 1958 there were 83 and 96, respectively. By 1961 this figure had risen to more than 260 in that one year alone. Apparently more than 400 articles per year can be expected for 1964 and 1965.

It would be virtually impossible to cite every article that refers to the use of immunofluorescence, but an attempt has been made in this six-volume annotated bibliography. Fifteen languages are represented, and more than 150 journals have been searched. Six abstracting journals have been included in the search. Translations were provided by several co-workers, government translating services, and the compiler. The earliest entry is dated 1905; significant publications through 1962 are included. Subsequent entries are being compiled and will be incorporated into revisions of this bibliography. The additions will, no doubt, increase considerably the bulk of these volumes.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. The entire publication, Miscellaneous Publication 3, carries the title: "Immunofluorescence, an Annotated Bibliography." The subtitles for the six volumes are: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author.

Accession numbers in each volume were assigned to articles by tens to allow room for expansion in subsequent editions. Accession numbers within each volume are consecutive throughout that volume, so the volume number must accompany the accession number to identify an entry unmistakably. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in the volumes.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

The second parts of Volumes V and VI contain only references to articles printed in the other four volumes. As in the other volumes, the references are placed in subject categories, and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous longer form. For unmistakable identification, they are listed below.

DANS	a. 1-dimethylaminonaphthalene-5-sulfonic acid b. 5-dimethylamino-1-naphthalene sulfonic acid or its sulfonyl chloride form.
FIC	fluorescein isocyanate
FITC	fluorescein isothiocyanate
FTA	fluorescent treponemal antibody
FTA-200	a modification of the above based on serum dilution.
PAP	primary atypical pneumonia
PAS	para-aminosalicylic acid
PBS	phosphate-buffered saline
RB 200	a. lissamine rhodamine RB 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B d. sulphorhodamine B e. acid rhodamine B
TPFA	<u>Treponema pallidum</u> fluorescent antibody
TPI	<u>Treponema pallidum</u> immobilization

Generally, the citations follow the format prescribed by the second edition of "Style Manual for Biological Journals," American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations, Z39.5-1963, American Standards Association Incorporated, New York.

The compiler started collecting this information in 1959 while he was stationed at the U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland. Since his transfer to the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he has continued this work with the encouragement and support of both installations.

The information in these volumes was originally recorded on coded Keysort cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, U.S. Army Biological Laboratories, Frederick, Maryland, 21701, for improvement of the subsequent editions.

ACKNOWLEDGMENTS

This compilation would have been much more difficult if not impossible without the guidance, help, and encouragement of:

Dr. Harold W. Batchelor, who introduced the compiler to card-sorting systems;

The staff of Naval Medical Research Institute, who provided both support and personnel for assistance in this work;

My colleagues at the U.S. Army Biological Laboratories and the Walter Reed Army Unit at Fort Detrick and at the Naval Medical Research Institute in Bethesda, who volunteered their technical competence and supplied moral support.

Obviously, outstanding cooperation and assistance of librarians was required for this work. The staff of the Technical Library, Fort Detrick, under the direction of Mr. Charles N. Bebee, was continually patient, understanding, and essential for the entire period of compilation.

Another essential in the chain to final publication: Mrs. Madeline D. Warnock and her staff in the Editorial Branch at Fort Detrick have served as editors, of course, but also as confessors, encouragers, consciences, and the required driving force, all of which have brought this to its publication. The compiler is most grateful to Mrs. Warnock and her people.

ABSTRACT

This volume is one of a series of six annotated bibliographies on various aspects of immunofluorescence and its use. Citations cover the period 1905 through 1962. Volume I contains 337 annotated literature citations, arranged according to major subject areas, and a complete author index.

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I. ACTINOMYCETALES

10

Arai, T.; Kuroda, S.; Ito, M. 1962. Possible utility of a fluorescent antibody technique in the serological identification of antagonistic Streptomyces. J. Bacteriol. 83:20-26.

The indirect fluorescent antibody technique was extended to the serological investigation of antagonistic Streptomyces. Serological relationship was studied among groups of antagonistic Streptomyces that are related in their ability to produce antibiotics or in their morphological and cultural characteristics. It was also demonstrated that spores and sporophores are as satisfactorily stained as shake-cultured vegetative mycelia.

20

Ehrlich, R.; Ehrmantraut, H.C. 1955. Instrumental estimation of bacterial population by fluorescence microscopy. Appl. Microbiol. 3:231-234.

A method is described for the quantitative estimation of bacteria by means of fluorescence microscopy. The method is based on the quantitative determination of intensity of fluorescence emitted from bacteria stained with the fluorochrome coriphosphine. For more uniform and reproducible results amber-colored rigid vinylite slides were substituted for conventional microscopic slides.

30

Kirsh, D.; Shepard, C.C. 1961. Disruption of mycobacteria and their stainability by fluorescent antibody. Federation Proc. 20:16.

Tubercle bacilli and several other slowly growing mycobacteria did not stain distinctly with fluorescent antibody when tested as intact organisms. They stained very well, however, after they were broken open. Disruption was accomplished by vibration with glass beads, and 2 to 6 minutes of treatment opened them up, so that the protoplasm, measured by optical density at 265 mμ of hot PCA extracts, was almost completely released and was no longer sedimentable at 1500 x g. The 1500 x g sediment accounted for about 20 per cent of the dry weight and 0.5 per cent of the protoplasm of whole organisms. It contained the cell walls as rod-shaped elements that stained brightly with FA. The 1500 x g supernate contained the balance of dry weight and protoplasm, and consisted of about 50 per cent protein. As measured by its ability to inhibit FA staining of cell walls, it contained more available antigen than the cell wall fraction or the intact bacilli. Its high antigen content was also attested by its titer as antigen in a CF test, and by its behavior in Ouchterlony plates, in which it gave more lines than culture filtrate concentrates. Thus the findings indicate that the outer surfaces of the cell wall of mycobacteria of these species are free of antigen.

10

40

Macotella-Ruiz, E. 1962. Immunological characteristics of Actinomycetes pathogenic for man as shown by fluorescent antibody. Derm. Tropica 1:175-183. In Spanish.

Taxonomic studies were done by standard methods. Seven species were serologically compared using FA and reciprocal adsorption tests. Five serologic groups were seen before adsorption: Actinomyces bovis, Nocardia asteroides and N. brasiliensis, Streptomyces madurae and S. pelletieri, S. somaliensis, and S. paraguayensis. Following adsorption the sera were specific.

50

Morris, J.A.; Aulisio, G.C.; Bozeman, F.M.; Guinto, R.S. 1961. Fluorescent antibody study of the human leprosy bacillus. Bacteriol. Proc. M83:123.

By application of the indirect fluorescent antibody technique, leprosy bacilli in impression films prepared from infected human spleen were found to combine with antibody present in most sera from lepromatous leprosy patients. Such sera when unabsorbed reacted also with Mycobacterium tuberculosis, but only rarely with the Binford bacillus and with acid-fast organisms recovered in hamsters injected with suspensions of human leprosy nodules. Lepromatous sera absorbed with M. tuberculosis lost their capability to react with this organism but still reacted with M. leprae. Sera from tuberculosis patients fluoresced with smears containing M. tuberculosis and M. leprae, but after partial absorption with the former organism, reacted only with smears containing M. tuberculosis. With application of a standardized procedure employing M. tuberculosis-absorbed sera, positive antibody tests were obtained in 11 of 14 sera from lepromatous leprosy patients and in five of 17 tuberculoid leprosy patients from the Philippines and in all eight sera from lepromatous patients in Japan. In 38 control sera from the Philippines, two gave positive results; both of these sera were from individuals with histories of prolonged contact with leprosy patients. Fluorescent antibody might be useful in serologic diagnosis of leprosy.

60

Nishihara, H.; Froman, S.; Weiner, H.E. 1959. Serial determination of serum glycoproteins and proteins in the early stages of tuberculosis. J. Lab. Clin. Med. 54:247-256.

Two major groups of antibodies were found in antisera produced in rabbits against human vascular endothelium extracts. One, reacting with serum proteins, could be removed by absorption with human serum. By precipitation, complement fixation, and other techniques, the remaining antivessel antibodies reacted against the specific endothelial antigens and extracts from kidney and spleen, which are highly vascularized organs, but not against liver. By the use of the fluorescein tagging technique, the antiendothelial antibody was localized, primarily at the level of the endothelial layer of the vascular intima.

70

Rotter, K.; Mayersbach, H. 1959. Investigations aimed at the demonstration of tissual antibodies in tuberculosis by means of immunohistological methods. Schweiz. Z. Allg. Pathol. Bakteriologie. 22:6:732-741. In German.

The problem of serologic specificity was particularly considered in this study. Tubercle bacilli were not stained by fluorescent sera obtained from diseased animals. Necrotic tissue also did not absorb TB serum.

80

Shepard, C.C.; Kirsh, D. 1961. Fluorescent antibody stainability and other consequences of the disruption of mycobacteria. Proc. Soc. Exp. Biol. Med. 106:685-691.

Mycobacteria were broken open by vibration with glass beads. Ninety to 95 per cent of the protoplasm and 70 to 80 per cent of the bacterial mass was released into a soluble state and could be separated by centrifugation from the sediment of disrupted bacilli, which consisted largely of cell walls. Intact bacilli of the five more slowly growing species studied were not stainable with fluorescent antibody, but disrupted bacilli stained brightly. This was interpreted as evidence that the outer surfaces of intact bacilli of these species are free of antigen. Intact bacilli of three of the more rapidly growing species stained well. The soluble fraction contained the greatest amount of antigen as judged by ability to inhibit staining of the disrupted bacilli. It could be used to absorb, or inhibit, cross-reacting antibodies to render immune sera more specific in their staining of disrupted organisms. The soluble fraction was a potent antigen in agar diffusion tests. In complement-fixation tests it was also an effective antigen with helpful specificity.

90

Slack, J.M.; Moore, D.W., Jr. 1960. Fluorescent antibody studies with Actinomyces bovis. Bacteriol. Proc. M176:142.

Actinomyces bovis ATCC 10048 was adapted to growth in a nonantigenic proteose-peptone dialysate medium. Rabbits provided titers of 1:640 to 1:2560. A globulin fraction of the antiserum was precipitated with ammonium sulfate. The globulin protein was determined, and it was conjugated with FITC at 0.05 mg per mg protein. A negative control and the test organism were included on slides; labeled normal globulins were included. Microaerophilic actinomycetes have been divided into serologic groups A, B, and C. Relationships have been reported between the Actinomyces and Corynebacterium, Lactobacillus, Propionibacterium, Nocardia, and Streptomyces. Labeled antisera were prepared for groups A, B, and C. Preparations gave the following results: Labeled A. bovis, 10048, antiserum produced fluorescence with only A. bovis and four Australian isolates labeled A. israeli. Group A antiserum produced fluorescence with A. bovis, groups A, B, C, and Corynebacterium acnes. Absorbed group B antiserum produced fluorescence only with group B, and group C with group C. There is an antigenic relationship between A. bovis 10048 and group A only. Labeled microaerophilic actinomycete antiserum gives fluorescence with C. acnes but not the species of Corynebacterium, Lactobacillus, Propionibacterium, Streptomyces, or Nocardia used.

Slack, J.M.; Winger, A.; Moore, D.W., Jr. 1961. Serological grouping of Actinomyces by means of fluorescent antibodies. J. Bacteriol. 82:54-65.

Serological groups A, B, C, and D of Actinomyces were established using fluorescent antibody. One hundred thirty-eight cultures were included in the study. Eighty-nine were classed in group A, 15 in B, 13 in C, and 21 in D. Isolates were from patients and animals with actinomycosis and from healthy human beings. There was no correlation between source of the isolate and serological group. Furthermore, no one species could be placed exclusively in one group, although the majority of those designated as Actinomyces bovis were in group A. Seventeen anaerobic diphtheroids and seven Corynebacterium acnes isolates were placed in group A. One diphtheroid was in each of groups B and D. On this basis it is suggested that these organisms be included in the genus Actinomyces. Additional species of Corynebacterium as well as Lactobacillus, Propionibacterium, Streptomyces, and Nocardia did not fluoresce with any of the group antisera.

White, R.G.; Coons, A.H.; Connolly, J.M. 1955. Studies on antibody production: IV. The role of a wax fraction of Mycobacterium tuberculosis in adjuvant emulsions on the production of antibody. J. Exp. Med. 102:83-104.

After injection of ovalbumin as a water-in-oil emulsion a pronounced adjuvant effect is demonstrable following the incorporation of tubercle bacillary wax into the oily phase of the mixture. With single doses of antigen, 10 mg of ovalbumin, there is a 4- to 5-fold increase in the amount of antibody at the median of 3-week serum levels in animals receiving a small, 40-ug dose of wax. With a 5-mg dose of wax there is an 8-fold increase in serum antibody levels at the median. A striking feature of the action of wax is the stimulation of a macrophage proliferation locally at the site of injection and the production of morphological abnormalities in these cells. As judged by staining techniques for antibody content, these locally assembled cells are not active in the formation of antibody. Wax injected in mineral oil results in a remarkable systematized stimulation of the reticulo-endothelial system.

II. BACILLACEAE

120

Biegeleisen, J.Z., Jr.; Cherry, W.B.; Skally, P.; Moody, M.D. 1962. The demonstration of Bacillus anthracis in environmental specimens by conventional and fluorescent antibody techniques. Amer. J. Hyg. 75:230-239.

Bacillus anthracis was recovered from 44.7 per cent of 76 samples of hair, dust, floor sweepings, etc. from a goat hair processing mill. The materials were washed in a detergent solution, the organisms concentrated by centrifugation after being heated to 70 C for 10 minutes, and samples of the sediment were inoculated into mice. Only two specimens yielded positive cultures when the same materials were washed in saline and samples placed on suitable media. On the other hand, 12 of 25 of the air samples taken in the same plant proved culturally positive. Fluorescein-labeled antiglobulin for B. anthracis was used successfully to shorten the time required for both presumptive and definitive identification of the anthrax bacillus.

130

Blagoveshchenski, V.A.; Kulberg, A.; Bulatova, T.I.; Korn, M. 1962. Preparation of a specific fluorescent anthrax serum. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:3:18-23. In Russian.

Anti-anthrax precipitating sera were fractionated, conjugated, and dialyzed. This product stained heterologous bacilli. Specific staining was obtained following Castellani's absorption methods using anthracoid and pseudo-anthrax bacilli. It was possible to successfully examine water or soil washings using this final product.

140

Bulatova, T.I.; Kabanova, Ye.A. 1960. Identification of the botulism pathogen with luminescent sera. J. Microbiol. Epidemiol. Immunobiol. 31:403-408.

The method for preparation and use of fluorescent globulin against C. botulinum, type B, cells is detailed. Specific fluorescence with C. botulinum, types A and B, is reported; types C, D, and E, in addition to C. perfringens, colon bacilli, and anthracoids, did not fluoresce. Some fluorescence was seen in certain strains of C. sporogenes. The reasons for the nonspecific reactions are discussed. The authors conclude that the method may be used successfully only for orientational identification.

Cherry, W.B.; Freeman, E.M. 1959. Staining bacterial smears with fluorescent antibody: V. The rapid identification of Bacillus anthracis in culture and in human and murine tissues. Zentralbl. Bakteriол. Parasitenk. 175:582-604.

Techniques suitable for the preparation of sera for encapsulated cells of B. anthracis were developed. Globulin labeled with fluorescein isocyanate was used for staining and identification of B. anthracis in cultures and in impression smears and sections of human and animal tissues. Application of the reagent to a cytological study of B. anthracis was demonstrated and discussed. When the specimen, culture, smear, or section became available, it could be stained and examined within a period of approximately one hour. Bacteria in paraffin sections of human tissues processed by the usual histopathological procedures were brightly fluorescent when stained with fluorescent antibody for B. anthracis. A contribution of the indirect fluorescent antibody test to determination of antibody in a patient's serum is discussed.

Crozier, D.; Woodward, T.E. 1962. Armed Forces Epidemiological Board: Activities of the Commission on Epidemiological Survey, 1961. Mil. Med. 127:701-705.

As a portion of brief reviews of the varied activities of the Commission, fluorescent antibody studies on P. tularensis, B. anthracis, VEE virus, and Rift Valley fever virus are reported. The first three organisms were identified from aerosols by the FA method, and the two viruses were detected in viremias, using tissue culture techniques in conjunction with FA.

Dowdle, W.R.; Hansen, P.A. 1961. A phage-fluorescent antiphage staining system for Bacillus anthracis. J. Infect. Dis. 108:125-135.

An indirect, or phage-fluorescent antiphage, staining system for Bacillus anthracis has been proposed and the immunologic specificity demonstrated. Phage-fluorescent antiphage-positive bacilli were readily identified by the characteristic appearance of the staining reaction, the particulate fluorescence being completely dissimilar to the usually uniform primary or secondary fluorescence. All 29 strains of B. anthracis examined gave positive phage-fluorescent antiphage reactions. Phage staining was more specific than conventional fluorescent staining. Of 91 saprophytic bacillus strains, 9 reacted with the phage-fluorescent antiphage system, whereas 43 were stained with fluorescent antibodies against the somatic antigens of B. anthracis and the spores from 42 were stained with fluorescent antibodies against the spore antigen of B. anthracis. The nine phage-fluorescent antiphage-positive nonanthrax strains consisted of five nonrhizoid variants of B. cereus var. mycoides and four strains of B. cereus. Highly encapsulated B. anthracis grown on bicarbonate medium under carbon dioxide did not stain with the phage system.

180

Ehrlich, R.; Ehrmantraut, H.C. 1955. Instrumental estimation of bacterial population by fluorescence microscopy. *Appl. Microbiol.* 3:231-234.

A method is described for the quantitative estimation of bacteria by means of fluorescence microscopy. The method is based on the quantitative determination of intensity of fluorescence emitted from bacteria stained with the fluorochrome coriphosphine. For more uniform and reproducible results amber-colored rigid vinylite slides were substituted for conventional microscopic slides.

190

Geck, P.; Szanto, R. 1961. Examination of Clostridium perfringens with the fluorescent tracer technique. *Acta Microbiol. Acad. Sci. Hung.* 8:423-425.

The applicability of fluorescent immunological staining for the rapid identification of Clostridium perfringens has been examined. The high-titered antibacterial serum was labeled with the fluorescent dye lissamine rhodamine RB 200. Homologous and control smears were stained directly and indirectly. About 50 C. perfringens cultures stained with the homologous labeled serum yielded preparations showing a bright orange fluorescence of plus 4 intensity. The numerous gram-positive and gram-negative control bacteria stained with the same serum gave no fluorescence. No fluorescence was obtained when C. perfringens was stained with rhodamine-labeled nonimmune rabbit serum. Spores of C. perfringens showed a fluorescence similar to that of the vegetative forms. The method was found suitable for the differentiation between the Clostridium types A and F. With adequately conjugated sera, the identification and typing of C. perfringens, which require several days and often 1 or 2 weeks by the usual methods, have been accomplished within one hour.

200

Glubokina, A.I.; Kabanova, Ye.A.; Levina, Ye.N.; Pishchurina, M.M. 1960. Technique of obtaining and applying, in microbiology, sera labeled with fluorescein isocyanate. *J. Microbiol. Epidemiol. Immunobiol.* 31:385-391.

This article is a review of the literature on the technique of use of fluorescent antibodies for staining bacteria. Detailed instructions are given for all aspects of serum handling, globulin precipitation, conjugation, elimination of nonspecific fluorescence, and staining of smears. Both the direct and indirect techniques with controls are described. Staining of B. anthracis and dysentery bacilli are specifically mentioned. The dye discussed is fluorescein isocyanate.

210

Kalitina, T.A. 1960. Detection of Clostridium botulinum by means of luminescent antibodies. *Bull. Exp. Biol. Med.* 49:278-280.

A conjugate of botulinus rabbit antiserum with fluorescein isocyanate was prepared. In staining films of pure cultures of C. botulinum, an intensive green luminescence

was obtained. When preparations of other anaerobic cultures, E. coli, staphylococci, or anthracoids were stained with the same conjugate, the specific luminescence was absent. It is possible to reveal solitary C. botulinum cells in the films prepared from suspensions with a concentration as high as 500,000 bacterial cells per ml.

220

Kampelmacher, E.H. 1960. Significance of the fluorescent antibody method in diagnosing anthrax. *Tijdschr. Diergeneesk.* 85:1802-1813.

Following a historical survey regarding diagnosis by means of fluorescent antibodies, the various possibilities of this test are discussed. The value of the method to anthrax diagnosis is examined while pointing out the difficulties that still exist in its practical execution.

230

Kuzmin, N.A. 1962. Absorbing sera for fluorescent analysis. *J. Microbiol. Epidemiol. Immunobiol.* 33:23-27.

Absorption of anti-anthrax conjugates using strains of B. anthracoides and pseudo-anthrax to produce specific conjugates is described.

240

Leonard, C.G.; Thorne, C.B. 1961. Studies on the nonspecific precipitation of basic serum proteins with gamma glutamyl polypeptides. *J. Immunol.* 87:175-188.

Efforts to identify antibodies to gamma-D-glutamyl polypeptide in sera from rabbits injected with capsulated cells of Bacillus anthracis were unsuccessful. The use of egg white lysozyme conjugated with fluorescent dye for staining encapsulated cells of B. anthracis is described. The reaction with egg white lysozyme can be used for the rapid detection of low concentrations of glutamyl polypeptide.

250

Levina, Ye.N. 1958. Fluorescein-labeled antibody for the detection of the anthrax bacillus: I. *J. Microbiol. Epidemiol. Immunobiol.* 29:6-11.

Precipitating anthrax sera labelled with fluorescein isocyanate were prepared and the results of this study show that it is possible to diagnose and identify anthrax from pseudo-anthrax and other gram-positive bacteria by its bright fluorescence.

260

Poetschke, G.; Uehleke, H.; Killisch, L. 1959. Studies with fluorescence-marked antibodies: V. Simultaneous demonstration of several antigens by different colored fluorescent antibodies. *Schweiz. Z. Allg. Pathol. Bakteriologie*. 22:5:758-765. In German.

The labeling of immune gamma globulins with the sulfochlorides of various fluorescent dyes is described. Sulfo-rhodamine B, a pigment with reddish-yellow fluorescence, proved to be a feasible contrasting agent for hydroxy-pyrene-trisulfonic acid chloride and other green-fluorescent labeling substances. P. morganii and B. cereus were used to demonstrate that simultaneous specific staining with a mixture of variously labeled antibodies is possible. The potential of the method, as well as its advantages and disadvantages, is discussed.

270

Pritulin, P.E.; Kuzmin, N.A. 1959. On the application of luminescent antibodies for accelerated diagnosis of anthrax. *Veterinaria* 36:7:69-75. In Russian.

Adsorbed tagged precipitating anthracic serum is a specific preparation for the detection of the pathogen of anthrax in objects of the outer habitat such as oats and hay and also for the identification of cultures. Because of the brilliant green-yellow luminescence of the capsules surrounding the microbial bodies, it is easy to detect B. anthracis in oats and hay within 1 to 1.5 hours. Because of intensive luminescence of the preparations treated with tagged serum and obtained from organs and tissues of animals that died of anthrax, the method of luminescing antibodies to be used for the diagnosis of anthrax in materials obtained from the cadavers of animals must be improved.

280

Riggs, J.L.; Seiwald, R.J.; Burckhalter, J.H.; Downs, C.M.; Metcalf, T.G. 1958. Isothiocyanate compounds as fluorescent labeling agents for immune serum. *Amer. J. Pathol.* 34:1081-1097.

Two fluorescent isothiocyanate dyes have been synthesized for use in labeling antibodies. The advantages of these isothiocyanates over the corresponding isocyanates depend upon their stability in a solid form, which allows their storage and use directly when needed. The substitution of a less toxic substance, thiophosgene, for the highly toxic phosgene gas offers added advantage. The dyes were successfully conjugated with various antisera. Specific staining of the following antigens was carried out, using the direct and indirect method: B. anthracis, P. tularensis, R. typhi, and adenovirus RI-67.

III. BEDSONIA (Miyagawanella)

290

Buckley, S.M.; Whitney, E.; Rapp, F. 1955. Identification by fluorescent antibody of developmental forms of psittacosis virus in tissue culture. Proc. Soc. Exp. Biol. Med. 90:226-230.

Developmental forms of psittacosis virus have been studied in mouse embryo liver tissue cultures in vitro by the fluorescent antibody technique. Inoculation of a large dose of virus resulted in the intracellular appearance of antigen in most of the outgrowing cells within 16 hours. A moderate dose of virus resulted in the focal distribution of specific fluorescence. When a small dose of virus was inoculated, no specific fluorescence was detected. The evidence indicates that virus antigen is present in the developmental forms within the cytoplasm of the infected cells throughout the various stages that make up the growth cycle of psittacosis virus.

300

Donaldson, P.; Davis, D.E.; Watkins, J.R.; Sulkin, S.E. 1958. The isolation and identification of ornithosis infection in turkeys by tissue culture and immunocytochemical staining. Amer. J. Vet. Res. 19:950-954.

Rapid etiological diagnosis of ornithosis in turkeys was accomplished without difficulty by culture of tracheal and cloacal specimens on yolk sac explants, with observation of the infected explants with fluorescent antibody. Although the yolk sac explant technique of Weiss and Huang yields monolayers of large entodermal cells that are well adapted to fluorescent antibody staining and microscopic observation, a cell culture procedure involving trypsinization with the development of monolayers from the resulting cell suspensions might be preferable. Preliminary trials indicate that satisfactory fluorescent antibody staining of developmental forms of stock Jo and 6BC strains of ornithosis virus can be carried out with Earle's I cells.

310

Donaldson, P.; Davis, D.E.; Watkins, J.R.; Sulkin, S.E. 1958. Tissue culture and fluorescent antibody techniques for rapid isolation and identification of agents of the LGV-psittacosis group. Bacteriol. Proc. M26:65.

Methods that might contribute to the rapid diagnosis of disease caused by members of the LGV-psittacosis group have been under investigation. Following preliminary studies with the agent of feline pneumonitis to devise the methods used, turkeys were experimentally infected with the Jo strain of ornithosis virus and cloacal and tracheal specimens were taken at intervals during the following month. Yolk sac explants on flying coverslips were inoculated with materials from these specimens and after two days' incubation were treated with fluorescein-conjugated turkey antiserum prepared against ornithosis virus. The demonstration of intracellular fluorescein-stained developmental forms was used as the criterion of infection. Explants infected with turkey materials displayed fluorescent antibody

staining forms differing from explants similarly infected with stock suspensions of ornithosis virus. Cloacal specimens were found to be more suitable than tracheal specimens and were usually positive as early as three days after inoculation of the turkeys. Tracheal specimens were often not positive as late as 21 days. Direct smears were difficult to evaluate because of the relative scarcity of intact cells containing typical developmental forms of the virus.

320

Nichols, R.L.; McComb, D.E. 1962. Immunofluorescent studies with trachoma and related antigens. *J. Immunol.* 89:545-554.

Methods are presented for the utilization of immunofluorescence in field and laboratory studies of the PLT antigens. Antigen-antibody reactions produced easily discernible fluorescence of PLT viruses in yolk sac and tissue culture. Elementary body inclusions in conjunctival scrapings from the eyes of trachomatous patients yielded a brilliant stain. Evidence for the specificity of these reactions is discussed, and the titration of PLT antisera by fluorescent antibody is presented. The superiority of fluorescent antibody to Macchiavello staining in the early detection of trachoma virus in egg isolation passages is detailed. A screening test for the presence of PLT antibodies in serum is described, based on the specific inhibition of immunofluorescence. The enhancement of serologic specificity of trachoma antiserum by cross-absorption with trachoma or LGV antigens is reported. These studies, if confirmed by others, constitute another step in the serologic differentiation of the psittacosis-lymphogranuloma venereum-trachoma group of viruses.

330

Ross, M.R.; Borman, E.K. 1962. Direct and indirect fluorescent antibody techniques for the psittacosis group of agents. *Bacteriol. Proc.* V74:147.

Direct and indirect fluorescent antibody techniques were developed for detection of psittacosis group antigen in infected tissue cultures and titration of group antibody in human antiserum. Growth of meningopneumonitis virus, MP, in fetal mouse lung cells was followed by infectivity and complement-fixing antigen titrations and cytological examination of FA-stained cultures. Although infectivity and CF antigen of 1:4 reached a peak in 2 days and remained constant for an additional 3 days, only cells tested 2 to 3 days after infection were suitable for FA staining because of artifacts in older cultures. Labeled rooster and guinea pig anti-MP and human antipsittacosis sera were titrated in direct FA and HI tests. Rooster serum showed brighter staining and higher antibody titers than guinea pig or human sera. FA reactions with 1 and 2 units of labeled rooster serum were inhibited by unlabeled rooster serum but without clearcut inhibition. Indirect FA technique was used for titration of group antibody in human serum. Indirect FA was intermediate in sensitivity between the HI and CF tests. None cross-reacted with human sera reactive for influenza A and B, parainfluenza 1, 2, and 3, respiratory syncytial virus, Q fever, or the PAP agent.

340

Siboulet, A.; Galistin, P.; Huriez, C. 1962. Preliminary notes on the advantages of the immunofluorescent technique for diagnosis of inclusion blennorrhea. Bull. Soc. Franc. Derm. Syph. 69:898-901. In French.

The rare diseases of nongonococcal urethritis and inclusion blennorrhea, less than 4 per 100, are contagious and serious in their complications. The present laboratory diagnosis is lengthy - biopsy, egg inoculation, and tissue culture. The immunofluorescent technique permits rapid diagnosis. The arguments given in this report for this technique help close a link in the chain for diagnosis of these two diseases.

350

Starr, T.J.; Pollard, M.; Tanami, Y. 1960. Cytochemical studies with psittacosis virus. Bacteriol. Proc. M81:112.

A virulent strain of psittacosis virus, Texas Turkey, was propagated in a tissue culture cell line of human amnion, Fernandez. Infectivity titers of culture fluids and cytochemical studies were made at time intervals after infection. Acridine orange, fluorescent antibody, feulgen, and conventional staining techniques were used to demonstrate the replication cycle. Preliminary results indicated that this cycle involved a predominantly DNA virus and an RNA matrix associated with host cytoplasm.

360

Starr, T.J.; Pollard, M.; Tanami, Y.; Moore, R.W. 1960. Cytochemical studies with psittacosis virus by fluorescence microscopy. Texas Rep. Biol. Med. 18:501-514.

Conventional staining techniques and fluorescence microscopy with acridine orange and with fluorescein-tagged antibody were used to study the response of human amnion and human synovial cell lines to infection with psittacosis virus. The yellow-green color reaction of acridine orange with cell constituents was considered DNA-staining, and rust-red color reaction RNA-staining. In response to infection with psittacosis virus, the host cytoplasm contributed an RNA-staining matrix around an initial DNA-staining particle. The development of this matrix involved a sequence of color changes from shades of red to orange to yellow to green with the subsequent emergence of mature virus. During the early stages of development, the RNA-staining matrix was removed with ribonuclease to reveal DNA-staining immature virus particles that were feulgen-positive but not antigenic to fluorescein-tagged antibody. Later stages of virus development were antigenic. Some antibiotics and fluorinated pyrimidines, which are known nucleic acid antagonists, interfered with the normal maturation process of psittacosis virus. Thus, this host-parasite differential staining system permits visual study of nucleic acid metabolism and offers a rapid, efficient means for assay of potential chemotherapeutic agents.

Vozza, R.; Balducci, D. 1961. The technique of fluorescent antibodies in ophthalmology: A study of herpes simplex vaccine keratoconjunctivitis, and human trachomatous infection. Amer. J. Ophthal. 52:72-77.

The fluorescent antibody technique was used to study experimental herpes simplex, vaccine keratoconjunctivitis, and human trachomatous infection. The technique proved to be effective in identifying cells experimentally infected by the virus. Inclusions stained intensely, demonstrating the presence of antigen at their level, as did giant herpetic cells. The results on trachomatous smears were uncertain. The presence of a soluble phase of the trachoma virus seems probable.

IV. BRUCELLACEAE

380

Biegeleisen, J.Z., Jr.; Bradshaw, B.R.; Moody, M.D. 1962. Demonstration of brucella antibodies in human serum: A comparison of the fluorescent antibody and agglutination techniques. *J. Immunol.* 88:109-112.

Brucella antibody titers were determined on 96 human serum specimens by the conventional tube agglutination and indirect fluorescent antibody tests. The agglutinin titers were routinely higher than those obtained by the fluorescence technique, but fluorescence always was observed when the sera contained antibodies demonstrable by agglutination tests. Comparisons were made of the titer obtained by both methods and their significance was discussed.

390

Biegeleisen, J.Z., Jr.; Moody, M.D. 1960. Genus specificity of fluorescein-labeled anti-Brucella melitensis globulin. *Bacteriol. Proc.* M172:140-141.

Failure to isolate B. melitensis from specimens is not uncommon and serological diagnosis is time-consuming. Rabbit antisera were prepared to intravenous injections of phenolized, whole-cell antigens of each of the three species of Brucella. Samples of the globulins obtained by ammonium sulfate fractionation of the immune sera were labeled with two different fluorescein isothiocyanate compounds. Duplicate heat-fixed smears of brucellae and heterologous strains were stained with twofold dilutions of each labeled anti-Brucella and normal globulin. Fifty-eight strains of Brucella stained with greatest fluorescence intensity at the highest dilution when labeled B. melitensis globulin was used. Seventy-eight heterologous strains gave no fluorescence that would interfere with specific demonstration of Brucella organisms. No fluorescence was observed in control smears. The results indicate that this conjugate is specific for the genus Brucella and might be used for the rapid identification of Brucella in dried smears.

400

Biegeleisen, J.Z., Jr.; Moody, M.D. 1961. Demonstration of Brucella antigen in tissue impression smears by the fluorescent antibody technic. *Bacteriol. Proc.* M89:124.

A fluorescein isothiocyanate-labeled globulin fraction of Brucella suis antiserum stained specifically 58 strains of B. abortus, B. melitensis, and B. suis in dried smears. No significant fluorescence was observed in 76 smears of heterologous strains. This conjugate stained brucellae in tissues of animals experimentally infected with strains of three Brucella species. Brucellae and areas of soluble antigen concentrations were stained specifically in impression smears of the liver and spleen. Controls were elimination of fluorescence in the fluorescence inhibition test using conjugate mixed with anti-B. suis serum, failure to demonstrate specific fluorescence in smears treated with normal globulin conjugate, and the absence of significant fluorescence in smears of organs from animals inoculated with

the diluent. The infected animals died from 2 to 10 days later. More bacterial cells and fewer areas of soluble antigen were observed in those animals dying soon after inoculation. As the length of the disease increased, fewer organisms but more areas of soluble antigen were detected. Demonstration of Brucella antigen in tissues of infected animals facilitated a rapid, strongly presumptive report of brucellosis.

410

Biegeleisen, J.Z., Jr.; Moody, M.D.; Marcus, B.B.; Flynt, J.W. 1962. The use of fluorescein-labeled anti-Brucella suis globulin for demonstrating Brucella antigen in animal tissues. Amer. J. Vet. Res. 23:592-595.

The globulin fraction of Brucella suis antiserum labeled with fluorescein isothiocyanate stained 58 cultures of Brucella abortus, Brucella suis, and Brucella melitensis but did not stain significantly 78 cultures of heterologous gram-negative and gram-positive bacteria. The fluorescent antibody direct and inhibition tests on impression smears of tissues of animals infected with Brucella spp., experimentally or naturally, indicate that this technique may be useful to demonstrate Brucella spp. or their antigens in animal tissues.

420

Elokhov, V.P.; Markelov, I.M.; Mukhin, V.F. 1959. Accelerated detection of the pathogens of certain diseases by the fluorescent antibody method. VoennaMed. Zh. 6:71-75. In Russian.

The highly specific and sensitive fluorescent antibody method is easily applicable to the quick detection of various agents. Most convincing is the use of the method for detecting agents in water and in washings from slightly contaminated surfaces, in which the percentage of positive results approximates 100. Satisfactory results have been obtained in the use of this method in studying heavily contaminated surfaces and certain food products. The agents of typhoid fever, brucellosis, and tularemia can be detected. Bacillary forms of bacteria with clearcut morphology can readily be diagnosed.

430

Carter, C.H.; Leise, J.M. 1957. Specific staining of various bacteria with a single fluorescent antirabbit globulin. Bacteriol. Proc. PH29:147.

This report describes the use of fluorescent-labeled antirabbit globulin for the detection of bacteria. The antirabbit globulin was prepared by the intravenous injection of methanol-precipitated globulin into a goat. This antirabbit globulin was conjugated with fluorescein isocyanate by the method of Coons and Kaplan. It was possible to stain bacteria in 15 minutes with this fluorescent globulin when the bacteria had been previously combined for 10 minutes with homologous rabbit antiserum. With bacteria exposed to heterologous rabbit antisera, no staining occurred. Positive reactions were obtained with Brucella suis, Pasteurella pestis, Pasteurella tularensis, and Vibrio comma, indicating the possibility of specifically staining a number of bacteria with only one fluorescent antiserum.

This indirect fluorescent staining method was compared with the direct method using specific fluorescent immune globulin for B. suis and for P. pestis. In each instance results with both the direct and indirect methods were comparable. Additional studies showed that small numbers of cells in both pure and mixed cultures could be detected with fluorescent antiserum.

440

Carter, C.H.; Leise, J.M. 1958. Specific staining of various bacteria with a single fluorescent antiglobulin. *J. Bacteriol.* 76:152-154.

A single fluorescent antiglobulin has been used in conjunction with specific nonfluorescent antiserum to stain specifically Brucella suis, Pasteurella tularensis, Vibrio comma, and Pasteurella pestis. Similar results were obtained in the direct procedure, which required a specific fluorescent antiserum for each of the organisms listed. Thus, the indirect procedure accomplished the same results with one instead of many fluorescent preparations. The indirect procedure was employed with both pure and mixed cultures and with small numbers of cells. It was possible to use successive applications of specific antisera and fluorescent globulin until the organism was identified. Cross-reactions were eliminated by employing low-titered antisera in both the direct and indirect reactions.

450

Cavanaugh, D.C.; Randall, R. 1959. The role of multiplication of Pasteurella pestis in mononuclear phagocytes in the pathogenesis of flea-borne plague. *J. Immunol.* 83:348-363.

Studies were made of the fate of virulent P. pestis inoculated into mice and guinea pigs by blocked fleas. Virulent P. pestis occurred in three types: S-type, phagocytosis-sensitive; R-type, phagocytosis-resistant and nonencapsulated; M-type, phagocytosis-resistant, well encapsulated, rich in fraction I. When the S-type was phagocytized by monocytes of normal animals, it multiplied and when released was the M-type. The plague-blocked flea inoculates the S-type. These cells are destroyed by polymorphonuclear leukocytes, but when ingested by monocytes, they multiply and become the M-type. This condition permitted the establishment of bubonic plague in hosts.

460

Chadwick, P.; Slade, J.H.R. 1960. Identification of bacteria by specific antibody conjugated with fluorescein isothiocyanate. *J. Hyg.* 58:147-156.

Reaction of slowly growing pathogenic bacteria with specific fluorescent antibody provided a means for their rapid identification. Several other isothiocyanates and sulphonyl chlorides tested were inferior to fluorescein isothiocyanate. Tests were performed by taking impressions of bacterial microcolonies on glass coverslips, drying, fixing, staining, washing, and mounting them. The preparations were then examined microscopically. Individual bacteria were sharply defined and stained at the periphery. Microcolonies of bacteria stained with normal labeled serum, or

heterologous labeled antiserum, showed either very dim fluorescence or no fluorescence. The technique permitted specific identification of B. suis, P. pestis, and P. tularensis within 20 hours of inoculation of agar plates with material suspected of containing one of these organisms.

470

Crozier, D.; Woodward, T.E. 1962. Armed Forces Epidemiological Board: Activities of the Commission on Epidemiological Survey, 1961. Mil. Med. 127:701-705.

As a portion of brief reviews of the varied activities of the Commission, fluorescent antibody studies on P. tularensis, B. anthracis, VEE virus, and Rift Valley fever virus are reported. The first three organisms were identified from aerosols by the FA method, and the two viruses were detected in viremias, using tissue culture techniques in conjunction with FA.

480

deRepentigny, J.; Frappier, A. 1956. Studies on H. pertussis liquid cultures: III. Localization of surface antigens by means of fluorescent antibody. Can. J. Microbiol. 2:677-683.

The fluorescent antibody technique was used in an attempt to clarify the relationship between the morphology of the surface of Haemophilus pertussis and the antigenic properties of its capsular antigens. First, specific antibodies were produced by injection into rabbits of agglutinogenic and protective surface washings of the bacilli. Then, these antibodies were made fluorescent and used to mark specifically and in situ the original capsular antigens at the surface of bacilli grown in liquid as well as on solid media. Thus was obtained morphological and specific evidence for the presence of a capsule containing antigenic material.

490

Donaldson, P.; Allen, S.J. 1960. Kupffer cell reactions in active anaphylaxis in mice induced with the aid of Bordetella pertussis. Bacteriol. Proc. M6:88.

The mechanism and organs involved in active anaphylaxis of mice require definition. For these experiments mice were sensitized by means of antigen combined with Bordetella pertussis. Following subsequent challenge and anaphylactic death of these animals, frozen sections of various organs were stained with fluorescent antibody against the antigen used. Kupffer cell hyperplasia and generalized fixation of the challenge antigen by these cells were found in the liver sections. Mice inoculated with the same amount of antigen alone but similarly treated otherwise showed neither the generalized fluorescent antibody staining of the Kupffer cells nor the apparent increase in numbers. The occurrence of islands of histiocytes in the livers of mice treated with pertussis vaccine was confirmed in these studies, but antigen uptake by these cells was not shown. No difference was observed in antigen fixation by cells of the adrenal, kidney, spleen, or thymus in sensitized mice as compared with control animals. Mice sensitized by multiple injections of larger amounts of antigen also showed Kupffer cell proliferation and antigen fixation.

500

Donaldson, P.; Whitaker, J.A. 1960. Diagnosis of pertussis by fluorescent antibody staining of nasopharyngeal smears. *Amer. J. Dis. Child.* 99:423-427.

Nasopharyngeal specimens were collected from children showing clinical signs and symptoms of whooping cough, from other patients, and from well persons. Smears were prepared and stained with fluorescent antibody specific for Bordetella pertussis. Of 36 pertussis patients 31 yielded smears in which the etiologic agent was immunochemically stained in numbers that varied roughly from three organisms per 50 microscopic fields to hundreds of organisms per field. From all of 13 pertussis patients who had been treated with antibiotic less than 48 hours before, smears were obtained in which the etiologic agent could be identified, but only two of 18 such patients whose period of antibiotic therapy was more than 48 hours showed an identifiable agent. Two of the eight persons who remained well but were exposed to siblings with pertussis carried B. pertussis, according to observation of their fluorescent antibody-stained smears. Two persons who later developed clinical whooping cough were likewise positive. All of 36 specimens from patients with other diseases were negative.

510

Eigelsbach, H.T.; Tulis, J.J.; McGavran, M.H.; White, J.D. 1962. Live tularemia vaccine: I. Host-parasite relationship in monkeys vaccinated intracutaneously or aerogenically. *J. Bacteriol.* 84:1020-1027.

As a portion of this study, FA techniques were used to trace P. tularensis in the lungs following aerosol exposure.

520

Eldering, G.; Eveland, W.C.; Kendrick, P.L. 1961. Fluorescent antibody staining and agglutination reactions in a study of Bordetella pertussis cultures. *Bacteriol. Proc.* M88:124.

In line with observations with antisera for other bacterial species, antisera that agglutinate B. pertussis cultures will usually show fluorescent staining with the same cultures when appropriate techniques are used. However, adsorbed sera specific for particular B. pertussis factors (antigens) failed to show FA staining with cultures possessing the factors. This was true with direct FA methods using whole serum, the precipitated globulin fraction, or with various fractions separated in a DEAE column. The various fractions retained agglutinins for the appropriate cultures. On the other hand, antisera produced against certain rough B. pertussis cultures have no demonstrable agglutinins for any Bordetella cultures but showed positive FA staining with both rough and smooth B. pertussis cultures. Observations with antisera produced against heated B. pertussis suspensions added to the evidence that the same serum component is not responsible for agglutination and fluorescent antibody staining.

530

Eldering, G.; Eveland, W.C.; Kendrick, P.L. 1962. Fluorescent antibody staining and agglutination reactions in Bordetella pertussis cultures. J. Bacteriol. 83: 745-749.

Bordetella pertussis antisera produced with smooth cultures gave positive results in agglutination and fluorescent antibody tests with smooth B. pertussis cultures. Similar results were obtained with antisera produced with the related species B. parapertussis and B. bronchiseptica when each was tested with the respective homologous antigen. Cross-reactions occurred with some of the antisera and heterologous antigens but a positive agglutination test was not always correlated with a positive FA reaction, and the reverse was also observed. Adsorbed B. pertussis antisera specific for factors 2, 3, 4, and 5 agglutinated appropriate B. pertussis cultures, but gave negative FA reactions. Factor 1 antiserum prepared by adsorption with heated, 100 C, antigen gave positive results in both FA and agglutination tests. Rough B. pertussis cultures were inagglutinable, but were FA-positive when tested with unadsorbed antiserum produced with either smooth or rough cultures. Rough cultures were FA-negative with factor 1 serum. The results suggest that for B. pertussis different serum components may be responsible for agglutination and FA staining. The implications with respect to the protection-inducing antigen are discussed.

540

Eveland, W.C.; Marshall, J.D.; Silverstein, A.M. 1957. The specificity of bacterial identification by means of fluorescent antibody staining technique. Bacteriol. Proc. 650:44.

Previous studies by Coons have demonstrated the specificity of the fluorescent antibody staining technique in the study of soluble antigens, viral agents, and some bacterial species. Moody has applied this technique to the staining of bacteria on smears. This report describes an extension of these methods to gram-negative species to indicate the practicability of differentiating among these organisms. The nature and extent of cross-reactions observed both with a single genus and across genus lines were noted. Labeled antisera were employed against one or more species of the following genera: Brucella, Pasteurella, Proteus, Salmonella, Shigella, and Paracolonobacterium. Within a genus, smears of several strains were tested with labeled antisera against one or two of the strains. The extent of cross-reactive staining in every case was less intense than the staining of the homologous strain. Cross-reactions were noted consistently within the genus Brucella, but no cross-reactions were observed in testing five strains of Salmonella. Certain minimal cross-reactions across genus lines seem not to parallel known agglutination results.

550

Fleck, L.; Evenchik, Z. 1962. Latex agglutination test with Brucella antigen and antiserum. Nature 194:548-550.

The latex agglutination inhibition test described allows detection of minute quantities of Brucella antigen in biological materials. The fluorescent antibody method distinguishes between specific and nonspecific inhibition. Antigenic fractions of Brucella were studied.

560

Hudson, B.W.; Quan, S.F. 1960. Use of fluorescent antibody technique for detection of Pasteurella pestis in rodents. Trans. Roy. Soc. Trop. Med. Hyg. 54:599-600.

Fluorescent antibody examination of tissue smears of laboratory and field-trapped rodents was used in detecting Pasteurella pestis.

570

Hudson, B.W.; Quan, S.F.; Kartman, L. 1962. Efficacy of fluorescent antibody methods for detection of Pasteurella pestis in carcasses of albino laboratory mice stored for various periods. J. Hyg. 60:443-450.

FA was used to detect plague antigens in infected mouse carcasses stored at various temperatures for various periods. FA was compared with blood agar plate culture and animal inoculation when viable bacilli were presumed present, and with Larson's Ascoli test for soluble antigens when plague bacteria were presumed to be nonviable. FA was superior to animal inoculation or culture in detection of P. pestis antigens in mouse carcasses stored for 4 days or longer at 37 C, for 8 days or longer at 20 to 25 C, and for 3+ days or longer at 4 to 5 C. The FA test and precipitin tests were equal for detection of plague antigens in carcasses stored for 16 to 82 days at 37 C and for 16 to 82 days at 20 to 25 C. Ten of 21 mice stored for longer periods, 140 and 340 days, at 4 to 5 C were negative by precipitin tests, whereas 19 were positive by FA. After 7 years at room temperature, tissues of two plague-infected guinea pigs were negative by Larson's modified Ascoli test but positive by FA.

580

Jaeger, R.F.; Spertzel, R.O.; Kuehne, R.W. 1961. Detection of airborne Pasteurella tularensis using the fluorescent antibody technique. Appl. Microbiol. 9:585-587.

Considerable research has been directed toward the development of rapid methods for the identification of airborne microorganisms. The application of the fluorescent antibody technique coupled with the impaction of contaminated air onto glass slides affords a rapid and specific method for the identification of airborne Pasteurella tularensis. Early experiments presented problems of cross-reaction with organisms other than P. tularensis. These cross-reactions are eliminated by specific adsorption and proper dilution of the conjugate. A series of experiments conducted under rigidly controlled laboratory conditions indicates that fewer than ten viable P. tularensis per slide can be detected by this method. Time of impaction as well as the presence of large concentrations of other microorganisms did not alter this number. Calculations indicate that a concentration as low as one viable organism per 5 liters of air can be detected.

590

Janney, G.C.; Berman, D.T. 1962. Staining intracellular Brucella organisms by means of fluorescent antibodies. Amer. J. Vet. Res. 23:596-598.

A Brucella fluorescent antibody preparation specifically stained Brucella abortus cells and accumulations of antigen within guinea pig peritoneal exudate cells maintained in tissue culture.

600

Kaufman, L.; Cherry, W.B. 1960. Influence of residual ammonium sulfate on the preparation of Brucella abortus fluorescent antibody. Bacteriol. Proc. M167:139.

Brucella abortus antiserum from rabbits was fractionated with half-saturated ammonium sulfate and samples were dialyzed for varying times. Protein analyses of nondialyzed and of 2-, 4-, and 24- to 72-hour dialyzed samples were made by the biuret method. High concentrations of ammonium sulfate interfered with the biuret determination of protein but not with agglutinating potency. Identical samples of the globulin fractions were conjugated to fluorescein isothiocyanate. Examinations of conjugates before and after dialysis revealed that total protein values by the biuret method at 540 mμ deviated from direct Kjeldahl determinations and from estimated biuret measurements of nonconjugated globulin. Total protein values measured at 560 mμ showed no significant deviation from estimated biuret and measured Kjeldahl values. Conjugation failed to alter protein content or agglutinating potency. High concentrations of ammonium sulfate adversely affected staining titers. Dialysis of 5- to 10-ml samples of globulin containing 1 to 2 grams per cent protein against two liters of physiological saline for 2 hours obviates ammonium sulfate interference with the protein determination by the biuret method and with the fluorescein labeling process.

610

Kendrick, P.L.; Eldering, G.; Eveland, W.C. 1960. Application of fluorescent antibody techniques to the diagnosis of pertussis. Bacteriol. Proc. M171:140.

Nasopharyngeal specimens collected by pernasal swabs have been examined by culture on Bordet-Gengou medium in comparison with direct slide preparations stained with specific antiserum conjugated with fluorescein isothiocyanate. Preliminary data from currently collected specimens correlated with clinical histories indicate that the staining procedure has specificity, gives a high percentage of positive findings, and is practicable wherever appropriate equipment is accessible. Consideration is being given the use of mailed specimens. It is possible with the use of fluorescent-stained antibodies to differentiate B. pertussis from the related species B. parapertussis and B. bronchiseptica. Also, application of the method to the study of the different serum factors of Bordetella is under investigation.

620

Kendrick, P.L.; Eldering, G.; Eveland, W.C. 1961. Fluorescent antibody techniques: Methods for identification of Bordetella pertussis. Amer. J. Dis. Child. 101:149-154.

Fluorescent antibody staining methods have real promise as an aid to identification of B. pertussis in direct nasopharyngeal preparations. The divergent results, though small in number, emphasize the need for comparing any new method with a standard accepted procedure - in this case the culture method. Through this comparison, certain limitations and pitfalls were revealed. For reliable results, familiarity with the infective agent is required, technical factors related to all steps in the procedure must be strictly controlled, and use of a suitable antiserum is essential. Further work is needed to establish criteria for defining an antiserum as to its sensitivity and specificity in diagnostic tests. It is recommended that diagnostic laboratories with the required equipment use the fluorescent antibody staining procedures, and thus add to the data needed as a basis for evaluation of its place in the laboratory identification of B. pertussis.

630

Levina, Ye.N.; Neimark, F.M. 1960. Detection of Haemophilus pertussis by the fluorescent antibody technique. J. Microbiol. Epidemiol. Immunobiol. 31:779-784.

Fluorescence microscopy of preparations of 61 strains of Haemophilus pertussis stained with fluorescent pertussis antiserum revealed a marked fluorescence of the bacterial cells, particularly at their periphery. A similar fluorescence could not be observed in 18 strains of H. paraptussis stained in a similar manner or in 30 strains of other cocci and bacilli isolated from children suffering from whooping cough. The investigation described above showed that specific detection of H. pertussis is possible in microscopical preparations made from mixed cultures after treatment with fluorescent pertussis antiserum.

640

Marie, J.; Herzog, F. 1962. Rapid diagnosis of whooping cough by the fluorescent antibody technique: Demonstration of Bordetella pertussis in nasal mucus. Preliminary results. Bull. Acad. Nat. Med. Paris 146:417-421. In French.

Application of the FA technique permitted improvement of prophylactic measures, reduction of the frequency of complications, and reduction of hospitalization time of patients.

650

Marshall, J.D.; Hansen, P.A. 1960. Histobacteriology, the study of microorganisms in fixed tissue by fluorescent antibodies. Virginia J. Sci. 11:175-176.

The feasibility of using the fluorescent antibody technique to differentiate several closely related bacterial species in formalin-fixed paraffin-embedded tissues was studied. Animals were experimentally infected with Pasteurella anatipestifer.

P. multocida, P. novicida, P. pestis, P. pseudotuberculosis, and P. tularensis. Tissues were taken at autopsy and processed for routine histological examination. Unstained sections were treated with fluorescein-conjugated immune sera prepared against each species of Pasteurella and examined by ultraviolet microscopy. Organisms and masses of degenerative antigenic material within the tissues stained brightly when homologous serum was used but not when heterologous sera were used. In lesions containing 2 or more species of bacteria, differentiating the species under investigation was not difficult. Correlation between specific histological changes with a single species of bacteria was possible.

660

Marshall, J.D.; Hansen, P.A.; Eveland, W.C. 1961. Histobacteriology of the genus Pasteurella: I. Pasteurella anatipestifer. Cornell Vet. 51:24-34.

Pasteurella anatipestifer was identified by the fluorescent antibody technique in smears of exudate and in histologically processed tissues of infected ducks. The tissue responses elicited by P. anatipestifer were differentiated from those reactions due to other causes.

670

McElree, H.; Downs, C.M. 1961. The phagocytosis of Pasteurella tularensis by rat mononuclear cells as influenced by normal serums and various irritants. J. Infect. Dis. 109:98-106.

The phagocytosis of Pasteurella tularensis by mononuclear cells harvested from the peritoneal cavity of rats was influenced by the serum employed in the tissue culture medium and the irritant used to elicit the exudate. Guinea pig and calf serum enhanced phagocytosis. Homologous serum, rabbit serum, and horse serum failed to enhance phagocytosis significantly or suppressed it. When peritoneal exudates were stimulated in normal rats by injection of either a peptone or a glycogen solution, the cells in such exudates exhibited an increased ability to ingest the organisms. However, cells obtained from peptone-treated rats suppressed the intracellular multiplication of tularensis bacilli as compared with the cells of the glycogen exudates, which allowed unrestricted multiplication of the organisms.

680

McGavran, M.H.; White, J.D.; Eigelsbach, H.T.; Kerpsack, R.W. 1962. Morphologic and immunohistochemical studies of the pathogenesis of infection and antibody formation subsequent to vaccination of Macaca irus with an attenuated strain of Pasteurella tularensis. Amer. J. Pathol. 41:259-271.

Monkeys were vaccinated intracutaneously with 100,000 viable cells of the living vaccine strain of P. tularensis. The bacteria multiplied locally, disseminated via the lymphatics to the regional lymph nodes and systemically to involve the liver and spleen. The bacteria evoked a mild, nongranulomatous and readily resolved inflammatory response. They disappeared from all the sites except the axillary and tracheobronchial lymph nodes between the 14th and 28th days. They

were present in these lymph nodes on the 28th but not on the 90th day. Anti-tularensis gamma globulin appeared in plasma cell precursors in the regional lymph nodes on the third day, in the spleen on the fifth, and in the dermis at the site of inoculation on the 14th day. ATGG persisted in the spleen and peripheral, or regional, lymph nodes through the 90th day.

690

McKeever, S.; Schubert, J.H.; Moody, M.D.; Gorman, W.; Chapman, J.F. 1958. Natural occurrence of tularemia in marsupials, carnivores, lagomorphs, and large rodents in southwestern Georgia and northwestern Florida. J. Infect. Dis. 103: 120-126.

Sera from 2004 mammals representing 13 species were tested for the presence of agglutinating antibodies of Pasteurella tularensis, and 344 had titers of 1:80 or higher. The hosts and incidence of infection were as follows: opossum, 11.9 per cent of 554; cottontail rabbit, 0.5 per cent of 188; fox squirrel, 2.5 per cent of 40; red fox, 11.5 per cent of 26; gray fox, 24.3 per cent of 136; raccoon, 24.9 per cent of 618; striped skunk, 21.5 per cent of 311; spotted skunk, 12.5 per cent of 8; wildcat, 22.0 per cent of 82; and feral house cat, 6.2 per cent of 32. One spleen, of 80 cultured, yielded P. tularensis. This opossum spleen culture was identified by agglutinin and fluorescent antibody reactions. Incidence of animal infection increased directly with their vegetation cover. All of the hosts are hunted and thus constitute potential sources of tularemia in man.

700

Meiselas, L.E.; Zingale, S.B.; Lee, S.L.; Richman, S.M.; Siegel, M. 1961. Antibody production in rheumatic diseases: The effect of Brucella antigen. J. Clin. Invest. 49:1872-1881.

Forty-one patients with various rheumatic diseases and 27 control patients were inoculated with Brucella vaccine. As a group the patients exhibited a significantly greater rise in anti-Brucella agglutinins compared with the controls. Some overlap in both groups was present. Alterations were noted in other antibody systems, Coombs anti-red cells, anti-thyroglobulin, and possibly in the influenza antibody and rheumatoid factor after this primary stimulation in some of the patients with rheumatic diseases, but no titers for these antibodies were noted in the control patients. The effect of Brucella antigen in these patients may be related to the damage that this organism can produce on mesenchymal tissue.

710

Merriott, J.; Shoemaker, A.; Downs, C.M. 1961. Growth of Pasteurella tularensis in cultured cells. J. Infect. Dis. 108:136-150.

Pasteurella tularensis strains of varying virulence have been cultivated in mouse fibroblasts and in beef kidney and human amnion cells cultured in vitro. The virulence of the organism used determined the ability of the organism to multiply or survive. Slight cytopathogenicity resulted from the intracytoplasmic multiplication

of P. tularensis in the L cell. The growth pattern of the organism in the L cell is influenced to a large extent by the type and amount of serum in the extracellular medium and by the nutrients present in the medium.

720

Mikhailov, I.F. 1961. Study of the properties of the antigen-antibody complex by means of fluorescent antibodies. J. Microbiol. Epidemiol. Immunobiol. 32:424-432.

The complex used included bacteria and specific antiserum. The antigen-antibody complex was firmly fixed by complement. On staining the antigens with fluorescent antibody, adsorption of the labeled antibodies took place. Unspecific conjugated proteins of the fluorescent antiserum were removed by subsequent washing. In the preparation of fluorescent antisera there is justification for the use of fractions of immune sera containing the maximal quantity of antibodies.

730

Moody, M.D.; Biegeleisen, J.Z., Jr.; Taylor, G.C. 1961. Detection of brucellae and their antibodies by fluorescent antibody and agglutination tests. J. Bacteriol. 81:990-995.

Conditions are described for preparing antigens and antisera and for performing three kinds of fluorescent antibody tests for detecting brucellae and their antibodies in various kinds of materials. The sensitivity and specificity of the fluorescent antibody tests were compared with those of cultural and agglutination techniques. Positive fluorescent antibody reactions were demonstrable with smears of suspensions containing as few as 2,500 viable or nonviable brucellae per ml. The presence of massive bacterial or environmental contamination did not appear to affect the sensitivity or specificity of the tests. Demonstration of positive agglutination reactions required suspensions containing no less than 60 million cells per ml. Higher serum antibody titers were obtained by agglutination and fluorescent antibody inhibition tests than by indirect fluorescent antibody tests.

740

Moody, M.D.; Thomason, B.M.; Winter, C.C.; Hall, A.D. 1959. Sensitivity of bacterial agglutination and fluorescent antibody reactions. Bacteriol. Proc. M103:89.

Antisera were produced in rabbits with killed antigens of Brucella species, Bacterium tularense, Pasteurella pestis, Malleomyces pseudomallei, Vibrio comma, and Group A Streptococcus. Suspensions containing varying numbers of organisms were tested by tube and slide agglutination with homologous antisera and in fluorescent antibody reactions. Similar suspensions mixed in varying ratios with a contaminant, Serratia marcescens, were also tested. Fluorescent antibody techniques were significantly more sensitive than agglutination tests. Agglutination reactions could not be demonstrated with suspensions containing fewer than 100 million organisms per ml. Positive fluorescent antibody reactions were demonstrated with suspensions containing as few as 40 to 100 organisms per ml. Clearcut agglutination reactions were obscured considerably in the presence of contaminants; fluorescent antibody reactions were unaffected. If specific fluorescent antisera can be produced for a given bacterial species,

fluorescent antibody offers the possibility of obtaining an early identification of a bacterial pathogen, since large numbers of organisms in pure culture are not necessary.

750

Moody, M.D.; Winter, C.C. 1959. Rapid identification of Pasteurella pestis with fluorescent antibody: III. Staining Pasteurella pestis in tissue impression smears. J. Infect. Dis. 104:283-294.

Plague infections in white mice were diagnosed at least 2 days earlier with fluorescent antiplague globulin than by conventional methods consisting of pure culture isolation and agglutination procedures. With fluorescent antibody, plague bacilli could be identified in tissue impression smears made within 2 days after injection of 250 bacilli per mouse, whereas other methods required 5 days. The time required for diagnosis was roughly inversely proportional to the number of organisms injected. The procedures described offer the advantages of utilizing the susceptible animal as a rapid culture medium for unknown specimens, and obtaining a more rapid diagnosis of plague in experimental animals than can be obtained by standard methods.

760

Page, R.H.; Caldronney, G.L.; Stulberg, C.S. 1960. Use of contrasting fluorescent conjugates for the identification of H. influenzae in cerebrospinal fluid. Bacteriol. Proc. M169:139-140.

In the bacterial meningitides, fluorescent antibody offers methods for rapid detection and identification of the etiologic agent. Spinal fluid smears from infants with Hemophilus influenzae meningitis, as well as with meningitis caused by other bacterial species, were studied. The globulin fraction of an antiserum to H. influenzae type B was conjugated with fluorescein isothiocyanate. This labeled antibody was either used alone or in combination with lissamine rhodamine RB 200 conjugated with heterologous serum fractions. The labeled H. influenzae antibodies reacted specifically with the homologous organisms but not with a variety of heterologous organisms. Spinal fluid smears stained with the fluorescein-tagged antibody alone caused no difficulty when the organisms were present in profusion. However, when spinal fluids were characterized by a marked pleocytosis together with a paucity of organisms, recognition of the latter was obscured by fluorescence of leukocytes and debris. This was overcome by use of the combination of specific and nonspecific conjugates, whereby leukocytes and debris reacted with the lissamine conjugate to provide a contrasting red background for the green fluorescence of specifically stained H. influenzae.

770

Page, R.H.; Caldronney, G.L.; Stulberg, C.S. 1961. Immunofluorescence in diagnostic bacteriology: I. Direct identification of Hemophilus influenzae in smears of cerebrospinal fluid sediments. Amer. J. Dis. Child. 101:155-159.

Fifty-three spinal fluid specimens, 50 from cases of acute meningitis and three from convalescent cases, were comparatively examined by fluorescein-labeled H. influenzae

occurred in 48 of these specimens. In the remaining five, the presence of fluorescent organisms in the spinal fluid smears was correlated with clinical evidence of H. influenzae meningitis and effective antibiotic therapy. The fluorescent antibody procedures within the limits of this study proved to be sensitive and specific and, when used in conjunction with conventional techniques, offered a solution to problems involved in the rapid and direct identification of microorganisms causing bacterial meningitis.

780

Redmond, D.L.; Kotcher, E. 1962. Studies on Haemophilus vaginalis. Bacteriol. Proc. M82:83.

Fluorescent antisera for strains of Haemophilus vaginalis and Haemophilus aegyptius were prepared and checked for specificity by direct and inhibition immunofluorescent techniques. Homologous and heterologous reactions were carried out with strains of H. vaginalis, H. aegyptius and H. influenzae, H. aegyptius, or H. vaginalis, Amies strain. With H. aegyptius antiserum, cross-reactions occurred with H. vaginalis. Cross-reactions with various organisms of the vaginal flora did not occur. Tube agglutination tests gave results similar to the immunofluorescent reactions. H. vaginalis, Amies strain, agglutinated with H. aegyptius antiserum, whereas Dukes, Edmunds, King, and U/L strains of H. vaginalis and H. influenzae did not. H. vaginalis, Amies strain, did not agglutinate in H. vaginalis, Dukes and King strains, antisera. Three groups of vaginitis patients were studied for the presence of H. vaginalis by wet mount for clue cells, cultural techniques, fluorescent antibody on vaginal smears, and gram-stained vaginal smears. A high positive correlation was found between culture and FA.

790

Riggs, J.L.; Loh, P.C.; Eveland, W.C. 1960. A simple fractionation method for preparation of fluorescein-labeled gamma globulin. Proc. Soc. Exp. Biol. Med. 105: 655-658.

A relatively simple method for separating fluorescein isothiocyanate-labeled gamma globulin from serum has been presented. The procedure has been applied to a number of labeled antibacterial and antiviral sera, and the labeled gamma globulin obtained gave definite specific fluorescence when used in the staining procedure with markedly low background fluorescence.

800

Riggs, J.L.; Seiwald, R.J.; Burckhalter, J.H.; Downs, C.M.; Metcalf, T.G. 1958. Isothiocyanate compounds as fluorescent labeling agents for immune serum. Amer. J. Pathol. 34:1081-1097.

Two fluorescent isothiocyanate dyes have been synthesized for use in labeling antibodies. The advantages of these isothiocyanates over the corresponding isocyanates depend upon their stability in a solid form, which allows their storage and use directly when needed. The substitution of a less toxic substance, thiophosgene, for the highly toxic phosphene gas offers added advantage. The dyes were successfully

conjugated with various antisera. Specific staining of the following antigens was carried out, using the direct and indirect method: B. anthracis, P. tularensis, R. typhi, and adenovirus RI-67.

810

Shantarenko, I.V. 1960. A study of para-agglutination by the fluorescent antibody technique. J. Microbiol. Epidemiol. Immunobiol. 31:2033-2036.

FA enables us to discern a specific agglutination reaction from an unspecific one. The para-agglutination test on slides or in tubes does not always represent an antigenic relationship even if absorbed type-specific agglutinating shigella antisera are used. Conclusions concerning the frequency of isolation of so-called atypical strains from objects of the external environment and human feces should be received with due criticism. Horse V. comma antisera O and P. pestis agglutinating antisera used at present are capable of reacting with some strains of E. coli and other bacteria, which must be kept in mind in attempts to detect bacterial contamination in the external environment using these antisera in the indirect fluorescent antibody technique.

820

Sinititskiy, A.A.; Diakov, S.I.; Mikhailov, I.F.; Nikitin, V.M.; Osipova, I.V. 1960. The use of the indirect method of staining Pasteurella pestis with fluorescent antibody: I. The specificity of the staining and the morphological features characterizing the fluorescence of Pasteurella pestis in vaccines. J. Microbiol. Epidemiol. Immunobiol. 31:2028-2032.

Indirect FA established that the organisms of plague vaccines 1-17, EB, and NIEG stain specifically. Treatment of smears with ordinary plague agglutinating antiserum allows the organisms in plague vaccines to be differentiated from other species with the exception of P. pseudotuberculosis. The specific fluorescence of the vaccine strain of P. pestis I-17 in the form of a yellow-green zone surrounding the cell decreased slightly on cultivating at 4 C, 18 to 22 C, and 37 C, but decreased considerably or disappeared when cultivated at 30 C.

830

Suzuki, S.; Furukawa, N. 1962. The application of fluorescent antibody technique in the field of pediatrics. Shonika 3:327-333. In Japanese.

This review article discusses the various FA techniques - direct, indirect, and complement-staining. It also describes how to prepare specimens, staining methods, and equipment for fluorescence microscopy. Clinical application of FA technique and diagnosis are considered in influenza, streptococcal infection, pertussis, diphtheria, and viral infections.

Tabakov, P.K.; Chibrikova, E.V.; Shurkina, I.I.; Velner, E.I. 1962. A rapid method of obtaining antibodies labeled with fluorescent stains. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:10:26-30. In Russian.

A simple method for antibody, or euglobulin, isolation from the agglutinating anti-cholera and antiplague sera has been suggested. A new method for purifying the fluorochrome-labeled antibodies from the excessive amount of the stain has been also presented. The employment of the mentioned methods reduces the time necessary for preparing the fluorescent conjugates to 3 or 4 days instead of 3 weeks, according to the classical Coons and Kaplan method; it does not require any special equipment, such as refrigerator centrifuges and special rooms. Soviet fluorochrome, or fluorescein isothiocyanate, was tested and the advantages of this preparation over isocyanate fluorescein were demonstrated.

Walker, R.V. 1962. Studies on the immune response of guinea pigs to the envelope substance of Pasteurella pestis: I. Immunogenicity and persistence of large doses of fraction I in guinea pigs observed with fluorescent antibody. J. Immunol. 88: 153-163.

According to fluorescent antibody observations, fraction I injected in oil persisted in guinea pigs for 186 to 210 days. Its distribution as detected with fluorescent antibody and by radioactivity differed. The latter suggested combination with antibody resulting in catabolism by the liver, elimination by the kidneys, and brief storage in bone marrow not seen with fluorescent antibody. Only fixed reticular macrophages and fibroblasts were observed to ingest the antigen. It was not stored long in these cells. Many vessels, sinuses, and other microscopic cavities were lined with a thick coating of the antigen. The last appearance in antibody-producing centers was in the germinal centers of Flemming. Partial immunity developed 3 months after primary injection and could be restimulated at 6 months. Immunounresponsiveness of guinea pigs to fraction I in oil was temporary. Eventually a partial immunity arose.

Walker, R.V. 1962. Studies on the immune response of guinea pigs to the envelope substance of Pasteurella pestis: II. Fluorescent antibody studies of cellular and tissue response in mice and guinea pigs to large doses of fraction I. J. Immunol. 88:164-173.

According to fluorescent antibody study of the distribution and fate of fraction I, it may be adsorbed by a variety of fixed tissues, such as endothelium of blood vessels, dead striated muscle or reticular tissue in spleen, lymph nodes, by fibroblasts, fixed reticulum cells in lymph nodes and bone marrow and fat cells. It may be transported by flowing freely in lymph or plasma, or as a thick coating on circulating orthrocytes or phagocytes. It is ingested by few mobile macrophages or microphages in a quantity detectable with this method. Its presence in Bowmans capsule and convoluted tubules suggests elimination in urine. Its last site of appearance in antibody-producing organs is the germinal centers of Flemming. Its prolonged presence as a thick coating

or lining in various structures suggests numerous areas of local excess that might absorb circulating antibody for a prolonged period, thus explaining the long-delayed immunity in guinea pigs given an excess of fraction I in oil adjuvant.

870

Walker, R.V. 1962. Studies on the immune response of guinea pigs to the envelope substance of Pasteurella pestis: III. Immunounresponsiveness to high concentrations of fraction I in oil. J. Immunol. 88:174-183.

Expanding on earlier studies, the causes for immunounresponsiveness in mice and guinea pigs to P. pestis fraction I in oil were studied. Variations in rapidity of excretion of fraction I, previous immunoexperience, and hyperimmunity all played roles. Complete and partial immunity and antibody plateau were investigated. Guinea pig immunounresponsiveness to fraction I in oil was apparently a temporary prolonged delay due to blockade of RES elements by the antigen.

880

Whitaker, J.A.; Donaldson, P.; Nelson, J.D. 1960. Diagnosis of pertussis by fluorescent antibody staining of nasopharyngeal smears. Amer. J. Dis. Child. 100:678-679.

FA methods were used to identify B. pertussis from clinical specimens by the direct method. The FA test was easier, cheaper, faster, and yielded more positives than did cultural methods. The conjugate stained S. aureus and B. bronchiseptica as well, but absorption with S. aureus cells removed this nonspecificity, and the staining of B. bronchiseptica was considered moderate.

890

Whitaker, J.A.; Donaldson, P.; Nelson, J.D. 1960. Diagnosis of pertussis by the fluorescent antibody method. New Eng. J. Med. 263:850-851.

Confirmation of clinical diagnosis of pertussis by the fluorescent antibody method was obtained in 94 per cent of 96 patients who had received no antibiotics and had been ill for less than three weeks. The incidence of positive fluorescent smears fell to 57 per cent in patients who had had a cough for more than 3 weeks and to 11 per cent in those who had received prior antibiotic therapy. Seventy-eight per cent of the total group of 128 patients with pertussis gave positive results. Control studies of healthy infants and children with other respiratory diseases indicate a high degree of specificity of the test. The clear superiority in all respects of the fluorescent antibody test over previous bacteriologic methods for the laboratory diagnosis of pertussis suggests that this rapid, specific, and simple test may replace the generally unrewarding cultural examination.

900

White, J.D.; Blundell, G.P. 1958. The use of the fluorescent antibody technic for demonstration of Pasteurella tularensis in formalin-fixed tissue. Bacteriol. Proc. PH12:136.

The presence of many pathogenic bacteria in infected tissues can be demonstrated by the application of selected dyes to sections of the fixed tissues. Pasteurella tularensis has been especially difficult to visualize by these conventional methods. It is generally accepted that in humans and in other higher mammals the organisms cannot be recognized by ordinary staining techniques. The fluorescent antibody technique of Coons is a valuable tool for the specific demonstration of antigenic material in various tissues. In the present study, the fluorescent antibody technique is used to recognize and identify P. tularensis in various tissues of macaca monkeys previously exposed to this pathogen. The tissue sections used in this report were selected from formalin-fixed material. Organisms were identified and localized in lung, spleen, and liver. Intracellular forms of P. tularensis were seen in macrophages of the lung and in Kupffer cells from the liver.

910

White, J.D.; McGavran, M.H.; Prickett, P.A.; Tulis, J.J.; Eigelsbach, H.T. 1962. Morphologic and immunohistochemical studies of the pathogenesis of infection and antibody formation subsequent to vaccination of Macaca irus with an attenuated strain of Pasteurella tularensis. Amer. J. Pathol. 41:405-413.

Twenty-four cynomolgus monkeys were vaccinated aerogenically with the living vaccine strain, LVA, of P. tularensis. The average inhaled dose was 270,000 viable cells. The bacteria initiated a mild, nongranulomatous inflammatory response in the respiratory bronchioles that was completely resolved by the 14th day after vaccination. LVA disseminated to involve the intrapulmonic lymphoid tissues, the tracheobronchial lymph nodes, the liver, and the spleen. By the 28th day all sites except the tracheobronchial lymph nodes were sterile, and no bacteria were recovered from these nodes on the 90th day. Anti-tularensis gamma globulin, ATGG, appeared in plasma cell precursors in the lung, about respiratory bronchioles, and in the peribronchial lymphoid tissues by the 7th day. By the 14th day mature plasma cells containing ATGG were prominent. The appearance of ATGG in the regional lymph nodes and spleen was like that found when dermal vaccines are used.

920

White, J.D.; McGavran, M.H.; Tulis, J.J. 1962. Immunohistochemical studies of antibody formation in monkeys vaccinated with an attenuated strain of Pasteurella tularensis. Bacteriol. Proc. M42:73.

The live vaccine strain of Pasteurella tularensis is one of the few effective viable bacterial vaccines. Using fluorescent antibody, we studied cellular formation of specific antibodies in cynomolgus monkeys vaccinated intracutaneously or by aerosol with an attenuated strain of P. tularensis, LVS. Localization of LVS was also studied by fluorescent antibody. Tissues were obtained sequentially after vaccination by biopsy or necropsy. Anti-tularensis gamma globulin, ATGG, was found first in the

regional lymph nodes of both groups, 3 to 5 days after vaccination. Five to 7 days after the appearance of ATGG in regional lymph nodes, ATGG was found in the spleen and other lymphatic tissues. Cellular ATGG was present in the lungs of aerosol-vaccinated animals 7 days after exposure but did not appear in the dermal inoculation site for 2 weeks. Cellular ATGG persisted in dermal and aerogenic vaccines through 90 days. Morphological differences between aerogenic and dermal vaccines were apparent only at sites of vaccination. Cytological and antibody response of these animals resembled a secondary response.

930

Winter, C.C.; Cherry, W.B.; Moody, M.D. 1960. An unusual strain of Pasteurella pestis isolated from a fatal human case of plague. Bull. WHO 23:408-409.

An unusual strain of P. pestis from a human case is discussed from the serological viewpoint. Fluorescent antibody studies provided a major clue to the bizarre nature of this strain because of its failure to stain with the whole-cell envelope conjugate preparation. Other peculiarities of the strain are discussed.

940

Winter, C.C.; Moody, M.D. 1957. Rapid identification of Pasteurella pestis with fluorescent antibody. Bacteriol. Proc. PH27:146-147.

Pasteurella pestis antiglobulin labeled with fluorescein isocyanate was used to stain P. pestis in dried smears from cultures and infected animal tissues. Antiserum was produced in rabbits, using formalin-killed whole cells from a virulent strain. The globulin fraction of serum was ammonium sulfate-precipitated. Individual cells in smears of P. pestis exhibited a bright fluorescence after treatment for 15 to 30 minutes with fluorescent antibody. Thirty strains of P. pestis reacted similarly; ten strains of Pasteurella pseudotuberculosis failed to stain. Ten strains of Shigella and one strain of Salmonella that were lysed by Pasteurella phage and 29 strains of other organisms isolated from soil, water, and sewage were not stained with P. pestis fluorescent antibody. P. pestis was detected in suspensions containing as few as 40 cells per ml, even when heavily contaminated with other bacteria. P. pestis was readily detected in impression smears. P. pestis was identified in bubo exudate and blood collected 4 days after onset of illness from a human case of bubonic plague. Organisms were detected occasionally when none could be cultivated. The results suggest the possibility of using this procedure as a reliable means of identifying P. pestis in a variety of materials.

950

Winter, C.C.; Moody, M.D. 1959. Rapid identification of Pasteurella pestis with fluorescent antibody: I. Production of specific antiserum with whole-cell Pasteurella pestis antigen. J. Infect. Dis. 104:274-280.

Antisera were produced to whole-cell, fraction I, and somatic substance of Pasteurella pestis. Group-specific and polyvalent sera to P. pseudotuberculosis were produced. These sera were tested by agglutination with available P. pestis and P. pseudotuberculosis strains to determine specificity. Agglutination tests showed that antiserum

produced with whole-cell P. pestis antigens was highly specific and equivalent to that produced with fraction I. The conditions required for producing a specific antiserum were growth of the organisms under conditions conducive to the synthesis of large amounts of fraction I and the use of a short immunization schedule. Cross-reactions between P. pestis and P. pseudotuberculosis antisera as reported in the literature were confirmed.

960

Winter, C.C.; Moody, M.D. 1959. Rapid identification of Pasteurella pestis with fluorescent antibody: II. Specific identification of Pasteurella pestis in dried smears. J. Infect. Dis. 104:281-287.

Globulin produced to Pasteurella pestis 16/P whole-cell antigen was labeled with fluorescein isocyanate. The specificity of the reagent was determined by testing with 100 strains of bacteria. Except for one virulent strain, Bryans, and two avirulent strains, TRU and 14, of the P. pestis, each of the 33 plague strains was stained brilliantly, provided that the cells had been produced under conditions that would enhance the production of fraction I. None of the P. pseudotuberculosis strains, nor 48 strains of other bacterial species, was stained with the reagent. The possibility of the use of this labeled globulin is discussed. A limited number of strains were also studied with a fluorescein-labeled anti-P. pestis somatic globulin. The study demonstrates the importance of evaluating and utilizing fluorescent antibody designed to detect specific antigens before it can be used for obtaining a reliable identification of P. pestis.

970

Wolochow, H. 1959. Fluorescent labels for antibody proteins: Application to bacterial identification. J. Bacteriol. 77:164-166.

A commercially available fluorescent compound, 5-dimethylamino-1-naphthalene sulfonyl chloride, which is relatively stable and does not require further chemical alteration, was shown to be a replacement for fluorescein isocyanate as a labeling agent for specific antisera when tested for identification of Pasteurella pestis. A commercially available light source, part of the Scopicon microprojector, may be easily adapted for use with fluorescent antibody techniques.

980

Yager, R.H.; Spertzel, R.O.; Jaeger, R.F.; Tigertt, W.D. 1960. Domestic fowl - source of high-titer P. tularensis serum for the fluorescent antibody technique. Proc. Soc. Exp. Biol. Med. 105:651-654.

A method for producing a high-titered, specific anti-P. tularensis globulin is presented. Roosters immunized with living P. tularensis organisms produced an antiserum that is more specific and has a higher working dilution than rabbit and monkey sera. The suspending medium for the organisms does not affect degree and intensity of specific staining or working dilution of serum. Cross-reactions obtained suggest that a specific antigenic relationship exists between P. tularensis and the Pseudomonas used in this study. This cross-reaction was eliminated by specific absorption with heterologous living organisms.

V. CORYNEBACTERIACEAE

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Dacres, W.G.; Groth, A.H., Jr. 1959. Identification of Erysipelothrix insidiosa with fluorescent antibody. J. Bacteriol. 78:298-299.

Two cultures of E. insidiosa from swine and one culture from a turkey were used. Heat fixation of the smears proved to be the method of choice. The slides were stained, washed, and mounted by the method described by Coons. Labeled antibodies were prepared against guinea pig complement for use in testing the complement-combining ability of the system. A 0.05 per cent solution of the conjugated protein was the lowest concentration that would stain E. insidiosa without visible loss of fluorescence. Guinea pig complement was not bound by the horse serum and E. insidiosa system when labeled antibodies prepared against the complement were used to demonstrate binding. Specificity controls included blocking, normal serum, and heterologous antigen.

1000

Jones, W.L.; Moody, M.D. 1960. Staining toxigenic Corynebacterium diphtheriae with fluorescent antitoxin. Bacteriol. Proc. M73:141.

The use of diphtheria antitoxin in direct and indirect fluorescent antibody tests to detect organisms in dried smears was investigated. Commercial diphtheria horse antitoxin was labeled with fluorescein isocyanate or isothiocyanate. Dried smears of viable suspensions of 19 Corynebacterium diphtheriae and 1 Corynebacterium ulcerans strain possessing toxin demonstrable in vitro and in vivo, and 7 C. diphtheriae and 5 diphtheroid strains shown to be atoxigenic were treated by direct and indirect FA. Eighteen of 19 toxigenic C. diphtheriae and one toxigenic C. ulcerans strain stained brilliantly by both techniques. One toxigenic strain failed to stain. Atoxigenic C. diphtheriae gave moderate staining reactions, but diphtheroids failed to react. Staining reactions of atoxigenic C. diphtheriae might be due to other antibody for certain bacterial antigens in the antitoxin. Staining of certain Staphylococcus aureus and beta hemolytic streptococci was demonstrated and may be the result of previous exposure to these organisms. The latter reactions were eliminated by absorption. Staining reactions were prevented by absorption with toxigenic C. diphtheriae. The reagents developed appear to be of value for identifying corynebacteria possessing diphtheria toxin, but results should be confirmed.

1010

Marshall, J.D.; Eveland, W.C.; Smith, C.W. 1959. The identification of viable and nonviable Erysipelothrix insidiosa with fluorescent antibody. Amer. J. Vet. Res. 20:1077-1080.

The fluorescent antibody technique as applied to Erysipelothrix insidiosa was investigated. The specificity of reaction was such that all 28 homologous strains tested were positively stained; there was no reaction in 140 of 150 heterologous strains. The exception was a weak staining reaction of one strain of Bacterium anitratum.

Chemical and physical treatments involved in histologic preparation of tissue specimens did not destroy the staining reaction. Erysipelothrix insidiosa was demonstrated in formalin-fixed, paraffin-embedded tissues.

1020

Marshall, J.D.; Eveland, W.C.; Smith, C.W.; Harr, J.R. 1959. Fluorescent antibody studies of Erysipelothrix insidiosa. Bacteriol. Proc. M106:90.

Failure to isolate this organism is not uncommon when other bacterial species are present. The use of the fluorescent antibody technique offers a method by which a greater number of cases can be diagnosed. Rabbit antiserum against E. insidiosa was prepared and conjugated with fluorescein isothiocyanate. When tested against 28 strains of the homologous organism and 150 strains of other bacterial species, all strains of E. insidiosa reacted but only one strain of Bacterium anitratum cross-reacted. The organism was studied in pure culture, mixed culture, and tissues of infected animals. When present in cultures, E. insidiosa was easily recognized, regardless of the presence of other species. The organism was demonstrated in tissue stained with a combination fluorescein-labeled homologous serum and a heterologous serum labeled with lissamine rhodamine RB 200.

1030

Moody, M.D.; Jones, W.L. 1960. Differentiation of diphtheria and diphtheroid bacilli with fluorescent antibody. Bacteriol. Proc. M187:145-146.

Antibody was produced in rabbits with O antigen, cells heated 2 hours at 127 C, and OK antigens, viable cells, of C. diphtheriae. Various K-serotypes, toxigenic and atoxigenic, gravis, intermedius, and mitis strains were represented among the six strains used. Globulin fractions were labeled with fluorescein isocyanate or isothiocyanate. Dried smears prepared from viable suspensions of 26 strains of C. diphtheriae and 6 strains of diphtheroids were fixed for one minute with 95 per cent ethanol, blotted dry, and stained by direct or indirect fluorescent antibody. All strains of C. diphtheriae were stained brilliantly by both methods by at least one of the 12 antisera tested. Similar cross-reactions occurred among the C. diphtheriae strains stained with either O or OK antibody. One strain consistently gave weak reactions. None of the diphtheroids was stained. Specificity and sensitivity in indirect tests were similar to those of direct tests. Toxigenic strains could not be distinguished from atoxigenic strains. All strains of C. diphtheriae tested, but none of the diphtheroids, could be identified in dried smears by using two selected antisera. The possibility of rapid detection of C. diphtheriae in clinical specimens is suggested.

1040

Smith, C.W.; Marshall, J.D.; Eveland, W.C. 1959. Investigation of Listeria monocytogenes by the fluorescent antibody technique. Bacteriol. Proc. M110:91.

Listeria monocytogenes has been implicated and suspected of being the etiologic agent in many important disease entities but, more often than not, cannot be properly studied because of the difficulty of isolating and characterizing it. As such an organism

can be of possible importance in clinical bacteriology, the use of FA was investigated as a tool in its study. Type-specific flagellar and somatic antisera were prepared in rabbits. A heterologous serum was prepared by combining equal parts of the type-specific sera. The sera were conjugated with fluorescein isothiocyanate. Human and animal isolates were studied in direct smears, mixed culture, impression smears, and tissue sections. A nonspecific serum conjugated with lissamine rhodamine RB 200 was used as a counterstain in impression smears and tissue sections to reduce nonspecific fluorescence. Of 30 strains tested, all gave specific fluorescence with either type-specific or a heterologous serum. In eye smears from laboratory-infected animals as well as in tissue sections, organisms could be demonstrated. Of more than 75 strains of bacteria screened with the heterologous serum, no cross-reactions were observed that could cause difficulty in specific detection.

1050

Smith, C.W.; Marshall, J.D.; Eveland, W.C. 1960. Identification of Listeria monocytogenes by the fluorescent antibody technic. Proc. Soc. Exp. Biol. Med. 103: 842-845.

Preparation is described of a polyvalent somatic fluorescein-labeled antiserum that was specific for 30 strains of Listeria tested but did not react with 180 heterologous strains. Preparation and specificity of flagellar and whole-cell antigens are discussed, as well as some technical implications of fluorescent antibody technique.

1060

Suzuki, S.; Furukawa, N. 1962. The application of fluorescent antibody technique in the field of pediatrics. Shonika 3:327-333. In Japanese.

This review article discusses the various FA techniques - direct, indirect, and complement-staining. It also describes how to prepare specimens, staining methods, and equipment for fluorescence microscopy. Clinical application of FA technique and diagnosis are considered in influenza, streptococcal infection, pertussis, diphtheria, and viral infections.

1070

Whitaker, J.A.; Nelson, J.D.; Fink, C.W. 1961. The fluorescent antitoxin test for the immediate diagnosis of diphtheria. Pediatrics 27:214-218.

An increase in the incidence of diphtheria stimulated search for a reliable laboratory test for immediate differential diagnosis of diphtheritic and non-diphtheritic membranous tonsillitis. Conjugated diphtheria antitoxin was highly specific for toxigenic C. diphtheriae. Eight of nine patients with proved diphtheria had fluorescent organisms on a smear of exudate. Failure in one case is attributed to poor quality of the specimen. An early case of diphtheria was detected by examination of family contacts of one of the patients. Conjugated antitoxin does not stain non-toxigenic diphtheria bacilli or other bacteria commonly found in the nasopharyngeal tract. Fluorescent staining of cultures suggests that only a small proportion of the potentially toxigenic diphtheria organisms are actually producing toxin at a given

moment. The fluorescent antitoxin test can be performed in one hour. A positive result is an absolute indication for antitoxin therapy. With a negative result the physician must still rely on his clinical judgment and cultural and virulence tests.

VI. ENTEROBACTERIACEAE

1080

Bals, M.; Zilisteanu, C. 1962. A method of orientation by means of fluorescent antibodies in the coprological diagnosis of typhoid fever. Rumanian Med. Rev. 6:15-17.

Typhoid was diagnosed from feces in 10 hours using FA. Demonstration of typhoid bacilli at 1:10,000 dilution of specimen was made. FA is recommended for parallel use in conjunction with classical procedures.

1090

Blokhov, V.P.; Markelov, I.M.; Mukhin, V.F. 1959. Accelerated detection of the pathogens of certain diseases by the fluorescent antibody method. VoennoMed. Zh. 6:71-75. In Russian.

The highly specific and sensitive fluorescent antibody method is easily applicable to the quick detection of various agents. Most convincing is the use of the method for detecting agents in water and in washings from slightly contaminated surfaces, in which the percentage of positive results approximates 100. Satisfactory results have been obtained in the use of this method in studying heavily contaminated surfaces and certain food products. The agents of typhoid fever, brucellosis, and tularemia can be detected. Bacillary forms of bacteria with clearcut morphology can readily be diagnosed.

1100

Cherry, W.B.; Thomason, B.M.; Pomales-Lebron, A.; Ewing, W.H. 1961. Rapid presumptive identification of enteropathogenic Escherichia coli in fecal smears by means of fluorescent antibody: 3. Field evaluation. Bull. WHO 25:159-171.

In field studies involving 315 children in Puerto Rico, it was demonstrated that presumptive diagnosis of infection with enteropathogenic E. coli could be made more rapidly and with greater sensitivity by immunofluorescence than by isolation and slide agglutination. The specificity of the fluorescent antibody test was of the same order as that of slide agglutination tests with OB antisera. The incidence of salmonellae, shigellae, coagulase-positive staphylococci, and Candida in the diarrheal specimens was studied, but no relationship appeared to exist between these organisms and the enteropathogenic E. coli. The value of fluorescent antibody techniques for the presumptive diagnosis of infantile diarrhea caused by E. coli and for monitoring institutional populations has been confirmed.

1110

Chucklovin, A.A.; Ivanova, S.P. 1961. Rapid diagnosis of bacterial dysentery with the aid of the fluorescent antibody method. *VoennoMed. Zh.* 9:55-57. In Russian.

The identification and typing of certain shigellae with fluorescent antibody were investigated. Comparisons with standard methods indicated good correlation. The time for this culture and FA combination technique was 16 to 18 hours, much shorter than standard methods. The applications to clinical situations were presented.

1120

Cohen, F.; Page, R.H.; Stulberg, J.S. 1961. Immunofluorescence in diagnostic bacteriology: III. The identification of enteropathogenic E. coli serotypes in fecal smears. *Amer. J. Dis. Child.* 102:82-90.

Fluorescein-labeled polyvalent and serotypic antibodies were used to detect and identify enteropathogenic E. coli serotypes 026:B6, 055:B5, 0111:B4, 0119:B14, 0124:B17, and 0127:B8 in fecal smears and smears of broth cultures of stool specimens from infants. Globulin fractions were also prepared to 065:B7, 0125:B15, 0216:B16. Labeled polyvalent antibodies were used to screen the specimens, and the individually labeled antibodies were used to identify the serotype. A total of 87 stool specimens was studied, 66 from infants with diarrhea due to EEC, and 21 from infants who had diarrhea of unknown etiology. A comparison of examination by fluorescent antibody and by conventional cultural procedures revealed that there was correlation between the two procedures in 76 specimens, 87.3 per cent. There was disagreement between the two methods in 11 instances, 12.7 per cent; in five specimens, 5.8 per cent, the fluorescent antibody methods failed to detect organisms originally isolated; in six specimens, 6.9 per cent, the FA tests detected EEC missed by cultural procedures. Combined use of labeled polyvalent and serotypic antibodies provided a rapid, sensitive method for early presumptive detection of enteropathogenic E. coli in fecal specimens.

1130

Cottrax, R.; Kass, E.H. 1962. Localization of bacteria antigens in experimental pyelonephritis: A fluorescent antibody study. *Federation Proc.* 21:24.

Reinfection and persistence of bacteria or bacterial antigens may be factors in the pathogenesis of chronic pyelonephritis. The localization of bacteria and fate of bacterial antigen were therefore studied in rat pyelonephritis by the fluorescent antibody technique. Acute and chronic pyelonephritis developed regularly after a single intravesical injection of B. proteus in rats. Animals were sacrificed 1 day to 13 months after infection and tissues were stained with specific antibody to B. proteus. Findings were correlated with bacteriological and histological data. Organisms initially invaded renal pelvic mucosa and spread through tubules and interstitium into the medulla and cortex. Bacteria were present within the kidney parenchyma in most infected animals up to 3 months after infection. Thereafter, bacteria were found in the pelvis, with renal calculi, and rarely in renal parenchyma proper. Specific antigenic material in macrophages of renal interstitium

persisted up to 13 months in most infected animals. Persistent infection was associated with active inflammation, i.e. neutrophils, pus casts, and plasma cells.

1140

Cremer, N.; Watson, D.W. 1957. Influence of stress on distribution of endotoxin in RES determined by fluorescein antibody technic. Proc. Soc. Exp. Biol. Med. 95: 510-513.

Deposition of endotoxin of Salmonella typhosa was followed in normal and stressed rabbits by the fluorescein tagging technique. Pretreatment with cortisone and X-irradiation did not affect initial phagocytosis of toxin but inhibited degradation and elimination of toxin by the RES. Pretreatment with thorotrast and preliminary injection of toxin caused depression of initial phagocytic functioning of the RES to a subsequent injection of toxin. Findings in their possible relation to the generalized Shwartzman reaction were discussed.

1150

Cremer, N.; Watson, D.W. 1957. Distribution and persistence of Salmonella typhosa endotoxin in rabbits. Bacteriol. Proc. M84:92.

Stressed and normal animals react differently to endotoxin. A comparison of distribution of toxin in such animals might suggest a primary site of action. Purified endotoxin of Salmonella typhosa was injected intravenously into normal rabbits and rabbits stressed with cortisone, thorotrast, X-irradiation, and a preliminary dose of toxin. After 20 minutes to 54 hours, the animals died or were killed. Frozen sections were prepared from liver, spleen, lung, kidney, heart, and adrenal and stained with S. typhosa-immune gamma globulin tagged with fluorescein. The major portion of toxin, when deposited, was found in the liver, spleen, and lungs with minimal amounts in kidney, heart, and adrenal. The most striking observation was failure of the RES of cortisone-treated and X-irradiated animals to destroy or dispose of phagocytized toxin when compared with the rapid removal within the RES of normal animals. Treatment with cortisone or irradiation did not impair ability to phagocytize the toxin. Thorotrast-treated animals and those receiving an initial injection of toxin exhibited a definite or complete inhibition of initial phagocytosis by their RES. There is no evidence that endotoxin localizes specifically at sites of major damage. Ability of the RES to take up or dispose of toxin modifies manifestations of endotoxin action.

1160

Dashkevich, I.O.; Diakov, S.I. 1959. Type-specific staining of Shigella flexneri using fluorescent antibodies. J. Microbiol. Epidemiol. Immunobiol. 30:53-58.

Fluorescent conjugates of S. flexneri C monoreceptor antiserum with fluorescein isocyanate have been prepared. These conjugates show a high degree of immunological specificity and intense fluorescence. A method has been developed for type-specific staining of S. flexneri that permits determination of the serological type in fixed films. The method of staining with fluorescent antibodies is highly sensitive and permits rapid identification of single cells of S. flexneri C on examination of preparations under the fluorescent microscope.

1170

Dashkevich, I.O.; Diakov, S.I.; Ermakov, N.V.; Ivanova, M.T.; Maiboroda, G.M. 1959. The staining of Salmonella typhosa with fluorescent antibodies. J. Microbiol. Epidemiol. Immunobiol. 30:126-133.

It was possible to prepare two types of fluorescent antiserum capable of specifically labeling S. typhosa and suitable for detection of these organisms in fixed preparations. Treatment with fluorescent antibodies is a highly sensitive method and enables S. typhosa to be quickly detected by fluorescence microscopy.

1180

Dashkevich, I.O.; Diakov, S.I.; Ermakov, N.V.; Ivanova, M.T.; Osipova, I.V. 1960. The use of the indirect fluorescent antibody technique for species-specific and type-specific staining of certain pathogenic bacteria. J. Microbiol. Epidemiol. Immunobiol. 31:2037-2043.

Fluorescent conjugates of chicken antirabbit gamma globulin labeled with fluorescein isocyanate were prepared that gave intense fluorescence and possessed high immunological specificity. A method for the species-specific and type-specific staining of certain pathogenic bacteria by the indirect fluorescent antibody technique was developed. It could be shown that in microbiological practice the indirect fluorescent antibody technique is superior to others in that it enables us to stain specifically various species of bacteria with only a single fluorescent antirabbit serum.

1190

Dashkevich, I.O.; Mikhailov, I.F. 1957. The production and use of fluorescent immune sera. J. Microbiol. Epidemiol. Immunobiol. 28:838-843.

The results show that the preparation and utilization of fluorescent antisera as a stain for enteric organisms are quite feasible. The substances obtained have a certain specificity in staining homologous bacteria. However, the brightness of the fluorescent bacilli after treatment with homologous fluorescent antiserum is still not sufficient for us to be able to use such substances for diagnostic purposes. Further work is required to improve fluorescent antisera and make methods more precise, so that they may be used for rapid diagnosis of bacterial contamination of materials.

1200

Davis, B.R.; Ewing, W.H. 1962. Serologic relationships that may lead to erroneous diagnoses of Escherichia coli infections by fluorescent antibody technics. Bacteriol. Proc. M78:82.

A study was made of intergroup antigenic relationships responsible for positive fluorescent antibody reactions obtained with cultures of various groups in labeled E. coli antisera. The majority of the strains investigated were submitted for serologic typing as E. coli because they gave positive FA reactions in labeled antisera for OB groups O26:B6, O55:B5, O111:B4, O127:B8, etc. The cultures were members of several

groups, such as Citrobacter, Klebsiella, and Providencia, and were found to possess O or K antigens related to those of the E. coli OB groups for which they were mistaken. These intergroup antigenic relationships, as well as previously reported intergroup relationships, may be expected to lead to erroneous diagnoses if results obtained by FA techniques are not confirmed by conventional biochemical and serological examinations. The results reported do not detract from the value of the FA technique as a method for presumptive identification of E. coli, but they do illustrate certain limitations of the method.

1210

Diakov, S.I.; Kasatkina, R.V.; Nikitin, V.M.; Pestryakova, Z.B. 1962. The use of fluorescent antibodies for rapid discovery of Shigella dysenteriae in milk. Gig. Sanit. 27:10:59-62. In Russian.

Of 131 milk samples, 72.4 per cent were negative, 15.4 per cent weakly positive, and 12.2 per cent were positive by FA for Shigella dysenteriae. No samples were culturally positive, but enteric bacteria of similar antigenicity were found in some samples. On subculture of the 62 parastrains isolated, there was a reduction of agglutinative ability in dysenteric antiserum. Changes of fluorescence were also noted in subcultured strains. Staining, when present, was generally nonuniform. It was felt that staining peculiarities in parastrains were sufficient for differentiation from dysentery bacilli.

1220

Ehrlich, R.; Ehrmantraut, H.C. 1955. Instrumental estimation of bacterial population by fluorescence microscopy. Appl. Microbiol. 3:231-234.

A method is described for the quantitative estimation of bacteria by means of fluorescence microscopy. The method is based on the quantitative determination of intensity of fluorescence emitted from bacteria stained with the fluorochrome coriophosphine. For more uniform and reproducible results, amber-colored rigid vinylite slides were substituted for conventional microscopic slides.

1230

Eveland, W.C.; Marshall, J.D.; Silverstein, A.M. 1957. The specificity of bacterial identification by means of fluorescent antibody staining technic. Bacteriol. Proc. 650:44.

Previous studies by Coons have demonstrated the specificity of the fluorescent antibody staining technique in the study of soluble antigens, viral agents, and some bacterial species. Moody has applied this technique to the staining of bacteria on smears. This report describes an extension of these methods to gram-negative species to indicate the practicability of differentiating among these organisms. The nature and extent of cross-reactions observed both with a single genus and across genus lines were noted. Labeled antisera were employed against one or more species of the following genera: Brucella, Pasteurella, Proteus, Salmonella, Shigella, and Paracolobactrum. Within a genus, smears of several strains were tested with labeled antisera against one or two of the strains. The extent of cross-reactive staining

in every case was less intense than the staining of the homologous strain. Cross-reactions were noted consistently within the genus Brucella, but no cross-reactions were observed in testing five strains of Salmonella. Certain minimal cross-reactions across genus lines seem not to parallel known agglutination results.

1240

Fleck, J.; Minck, R.; Kirn, A. 1962. Studies on the specific inhibitory activity of immune sera on bacterial L cultures: III. Localization of H antigen by the immunofluorescence technique. Ann. Inst. Pasteur Paris 102:243-246. In French.

The H antigen was demonstrated at the periphery of the large bodies and on flagellae of the stable L forms type 3A of proteus Pl8.

1250

Gelzer, J.; Suter, E. 1959. The effect of antibody on intracellular parasitism of Salmonella typhimurium in mononuclear phagocytes in vitro. J. Exp. Med. 110:715-730.

The effect of antibody on the fate of Salmonella typhimurium within mononuclear phagocytes, MN, of rabbits was studied in vitro. Monocytes and bacteria were incubated in absence or presence of antibody. Following washing, the cells were reincubated in a medium without addition of antibody, and the interaction between the MN and bacteria was followed. Anti-Salmonella antibody was not bactericidal even in the presence of complement and did not enhance phagocytosis. Regardless of whether antibody was present or absent during phagocytosis, the bacteria appeared to multiply within the cells. When no antibody was present during phagocytosis, the infected cells were damaged within a few hours of incubation, and extensive extracellular multiplication was dominating. When antibody was present, MN packed with bacteria persisted and little extracellular growth occurred. FA demonstrated antibody within the infected MN, appearing as a coat around the bacteria. Antibody entered cells only during phagocytosis. Active factor of immune serum was in gamma globulin and reacted specifically with the somatic antigen. The anti-flagellar portion of the antiserum was not involved. This antibody protects monocytes against the effect of intracellularly located Salmonella.

1260

Goldman, M.; Carver, R.K. 1961. Microfluorometry of cells stained with fluorescent antibody. Exp. Cell Res. 23:265-280.

The amount of fluorescein bound to protein was determined by spectrophotometric and fluorometric measurements of free dye occurring in the dialysate of a labeled conjugate, using the labeled compound fluorescein isothiocyanate as a standard. Two samples of fluorescein and one of fluorescein amine were unsuitable as standards for this purpose. The amount of bound dye could also be determined by spectrophotometric comparison of the conjugate with fluorescein isothiocyanate solutions. The average value for bound fluorescein was 54 ug of dye per mg of protein when the initial reaction mixture contained 100 ug of dye per mg of protein. When the initial mixture contained 50 ug of dye per mg of protein, the average amount of bound fluorescein was 23.8 ug. The fluorescence of bound fluorescein isothiocyanate was about 10 per cent

of that of the free dye in buffered saline solution. Microfluorometry of individual coliform bacteria showed that the average amount of antibody protein reacting with a cell was 1.15×10^{-7} ug carrying 3.25×10^{-9} ug of fluorescein.

1270

Gorokhev, P.D.; Rudey, Z. Kh. 1961. Experience in the use of fluorescent antibodies for the diagnosis of dysentery. *VoennoMed. Zh.* 9:59-60. In Russian.

Suspensions of colonies fished from an isolation medium were smeared on slides for FA studies and also identified by standard procedures. Cultures and slides were examined for Flexner dysentery bacteria. FA gave an accurate positive or negative result within 22 hours.

1280

Grabovskiy, P.M. 1961. The serological diagnosis of acute dysentery by the fluorescent antibody technique. *J. Microbiol. Epidemiol. Immunobiol.* 32:193-196.

The indirect Coons technique permits the detection of antibody in the sera of patients suffering from acute dysentery. The method in question is more sensitive than the slide agglutination test, although it is not sufficiently species-specific to permit discrimination between S. flexneri and S. sonnei. Fluorescent antibody cannot yet be recommended for large-scale introduction into laboratory practice.

1290

Hill, A.G.S.; Deane, H.W.; Coons, A.H. 1950. Localization of antigen in tissue cells: V. Capsular polysaccharide of Friedlander bacillus, type B, in the mouse. *J. Exp. Med.* 92:35-44.

The capsular polysaccharide of Friedlander B bacillus was traced following intravenous injection using the FA technique. It was rapidly absorbed by phagocytic cells throughout the body, persisting for more than 33 days. The substance was detected in capillary epithelium, collagenous fibers of all organs, lymphocytes of the spleen and lymph nodes, hepatic epithelium and bile, various portions of the kidney, the ovary, and the suprarenal cortex. In joints the polysaccharide was found in the synovial membranes, cartilage cells, osteoblasts, and osteocytes. When administered in aerosol, the pulmonary macrophages contained the polysaccharide.

1300

Ivanova, S.P. 1960. Identification of bacteria of the typhoid-paratyphoid group by means of the fluorescent antibody technique. *J. Microbiol. Epidemiol. Immunobiol.* 31:2016-2022.

This study showed that representatives of the Salmonella group, including S. typhi, could in principle be diagnosed by means of polyvalent fluorescent anti-S. typhi gamma globulin in aqueous suspensions and on the surface of various objects.

1310

Kabanova, Ye.A.; Glubokina, A.I. 1958. The use of antibodies labeled with fluorescein for the detection of dysentery bacilli. J. Microbiol. Epidemiol. Immunobiol. 29:3-6.

A fluorescent dysentery antiserum has been prepared that may be used to produce specific fluorescence in dysentery bacilli and to identify them in microscopical preparations.

1320

Kabanova, Ye.A.; Mordvinova, N.B.; Kuznetsova, N.S.; Mindlina, R.S.; Botvinnikova, M.E.; Mikhailova, Yu.M. 1960. The use of fluorescent antisera for the diagnosis of dysentery and coli enteritis. J. Microbiol. Epidemiol. Immunobiol. 31:2022-2027.

The immunofluorescence method is highly sensitive, sufficiently specific, easy to perform, and yields a rapid answer 1.5 hours after the beginning of the investigation if the smear is made directly from the sample in question without cultivation or after 18 hours if cultivation is carried out. The method can only be used for the diagnosis of infectious diseases of which the causative organisms have a sufficiently specific antigenic structure. Our investigations show that the method can be recommended for the diagnosis of conditions caused by pathogenic strains of E. coli 0111 and 055.

1330

Koeditz, H.; Weinert, A. 1960. Rapid identification of pathogenic E. coli with fluorescent antibodies. Kinderaerztl. Praxis 28:11:513-520. In German.

Information is given concerning a simple and fast method for the recognition of the dyspepsiocoli types 0111:B4, 055:B5 and 026:B6 by means of DANS-conjugated fluorescent antibodies. The authors could prove the presence of dyspepsiocoli in 435 of 700 samples of feces by means of the fluorescence method, although bacteriological serological tests run at the same time were positive in only 251 cases. The fluorescence method is therefore apparently a sensitive testing method, because it detects germs that do not multiply on the usual nutrients because of antibiotic therapy or that are present in such small numbers among other coli germs that they are not caught during agglutination. The special advantage of the fluorescence method is that it is possible to have the proof of the dyspepsiocoli germs within an hour after admission of the child, so that suitable isolation can prevent the spread of the dyspepsiocoli infection.

1340

Kozhushko, M.I.; Kozar, M.I.; Margulis, I.L. 1961. Diagnosis of bacterial dysentery by means of fluorescent gamma globulin. VoennoMed. Zh. 9:57-59. In Russian.

Fluorescent anti-Flexner bacillus globulin was used to identify dysentery bacteria in smears of stool suspension and scrapings from membrane filters through which stool suspensions had been passed. Results were confirmed by standard laboratory

methods. More positive results were obtained by FA methods than by culture. Flies experimentally infected with Flexner bacillus were also examined with positive results.

1350

LaBrec, E.H.; Formal, S.B. 1960. Studies of experimental bacillary dysentery by the fluorescent antibody technique. *Bacteriol. Proc.* M47:133.

FA was employed to study experimental Shigella infection in guinea pigs. Frozen sections from animals succumbing to oral challenge with Shigella flexneri 2a were prepared, fixed, and stained with fluorescein-labeled S. flexneri 2a antiserum and similarly labeled preimmunization serum. Shigellae were observed in and around focal necrotic lesions of the ileum and cecum of animals dying 20 to 28 hours after challenge; large numbers of these bacteria were within phagocytic cells. Small collections of S. flexneri were observed in the lamina propria of a solitary villus of the ileum with no apparent defect in the mucosal epithelium in the vicinity. Although many dysentery bacilli were seen in the lumen of the upper colon of these animals, lesions were not observed. More extensive changes were observed in animals dying 40 to 48 hours postchallenge. Shigellae were occasionally visualized in aggregate lymph follicles of the cecum adjacent to lesions. Dysentery bacilli were in lesions of the colon in animals dying at this later time. S. flexneri were not seen in the ileocecal lymph nodes, the mesenteric lymph node complex, or in organs outside of the alimentary tract in any guinea pigs. A few animals that survived 96 hours after challenge were sacrificed and no dysentery bacilli were demonstrated.

1360

LaBrec, E.H.; Formal, S.B. 1961. Experimental Shigella infections: IV. Fluorescent antibody studies of an infection in guinea pigs. *J. Immunol.* 87:562-572.

Sections of tissue obtained from guinea pigs succumbing to a fatal enteric infection of Shigella flexneri 2a were examined by fluorescent antibody and conventional histologic procedures to determine the nature and extent of a fatal infection. Shigellae were neither cultured nor visualized in any organs outside of the intestinal tract. In most of the animals studied that succumbed to infection at 24 hours, ulcerative lesions of the midileum and terminal ileum and cecum were noted. In animals dying 72 to 96 hours postchallenge, ulcerative lesions of the duodenum, jejunum, or midileum were never seen. These animals consistently had large ulcerations of the cecum and colon, whereas lesions of the terminal ileum were rare. In all of the challenged animals examined, shigellae could be found in the lamina propria of solitary villi without apparent loss of mucosal continuity. Examination of sections of intestine revealed that shigellae may invade the lamina propria of villi within 8 hours. Whenever ulcers formed they always contained specifically fluorescing dysentery bacilli, and in early stages they did not appear to be contaminated with other microorganisms.

1370

LaBrec, E.H.; Formal, S.B.; Schneider, H. 1958. Serological analysis of Shigella flexneri by the fluorescent antibody technique. Bacteriol. Proc. PH9:135.

The fluorescent antibody technique has been applied to the identification of Shigella flexneri serotypes. Rabbit antisera to Shigella groups A, B, C, and D as well as those against the individual S. flexneri types were conjugated with fluorescein isocyanate and tested for specificity by staining bacterial smears made from saline suspensions of the appropriate organisms. Results indicate that although group B serum stained all S. flexneri strains, certain of these cultures also reacted weakly with sera against groups A and C. Smears of S. flexneri stained with labeled, unabsorbed antiserum prepared against the various flexneri types exhibited the usual cross-reactions, but the intensity of fluorescence of heterologous cross-reactions was usually weak. However, when smears were flooded with unlabeled pooled flexneri group factor serum containing factors 3, 4, 6 and 7, 8, 9, prior to the use of labeled unabsorbed antiserum, the intensity of fluorescence of the heterologous types was extinguished or significantly reduced while retaining that of the homologous types. Identification of S. flexneri types using labeled, adsorbed, type-specific antiserum gave results as satisfactory as the slide agglutination technique.

1380

LaBrec, E.H.; Formal, S.B.; Schneider, H. 1959. Serological identification of Shigella flexneri by means of fluorescent antibody. J. Bacteriol. 78:384-391.

The fluorescent antibody technique has been employed to group and type Shigella flexneri in pure culture. Using direct staining, reactions were of the same specificity as those of the slide agglutination method. S. flexneri was stained by Shigella group B antiserum but not by antisera against groups A, C, or D. Similarly, by using labeled, adsorbed, type-specific S. flexneri sera it was possible to type S. flexneri cultures. The indirect staining method was not satisfactory because the available sheep antirabbit globulin contained normal antibody against various strains of Shigella. Attempts to identify S. flexneri in fecal specimens from infected guinea pigs were only partly successful. When labeled Shigella grouping sera were employed, diagnosis could not be made with any degree of accuracy when the number of Shigella organisms present was small since, on occasion, groups A and C antisera stained significant numbers of morphologically similar organisms in the feces of normal animals. Fecal smears from normal, healthy human volunteers contained microorganisms stained by the various sera. When labeled S. flexneri typing sera were employed, nonspecific staining was not observed.

1390

Larincov, A.P.; Kuzmin, N.A. 1959. The detection of the pathogens of paratyphoid toxoinfection by using fluorescent antibodies. Veterinaria 36:68-75. In Russian.

Fluorescent sera are applicable for the specific detection of paratyphoid bacteria in microscopic preparations. The use of labeled adsorbed sera is the fastest and most exact indication of paratyphoid bacteria in cultures and in pathologic material.

1400

Marrack, J. 1934. Nature of antibodies. *Nature* 133:292-293.

Breinl and Haurowitz showed that serum coupled with p-aminobenzenearsinic acid retained some of its specific agglutinating power. The proteins are connected to red azo dyes. Specific sera so treated were tested against their homologous antigens, typhoid bacilli, and cholera vibrios. The specific agglutination reactions were colored pink; reverse combination of the antigen-antiserum mixtures gave no color. The assumption was that the protein dye of the antiserum was specifically taken up by the bacterial antigen.

1410

Martineau, B. 1962. Fluorescent antibody in diagnosis of enteropathogenic Escherichia coli. *Can. Med. Ass. J.* 87:947-953.

FA was routinely used to detect enteropathogenic E. coli. The dyes FITC, rhodamine B isothiocyanate, and RB 200 were compared. Of 2061 stool specimens there were 61 positive FA but negative culture, and 33 positive culture but negative FA, using specific E. coli 0127:B8 antiserum. Of 457 stool specimens examined using polyvalent antiserum, there were 20 specimens negative FA but positive culture and 15 specimens positive FA but negative culture. Disagreements were respectively 4.6 and 7.6 per cent. FA offered rapid and sufficiently precise results.

1420

Mikhailov, I.F. 1961. Study of the properties of the antigen-antibody complex by means of fluorescent antibodies. *J. Microbiol. Epidemiol. Immunobiol.* 32:424-432.

The complex used included bacteria and specific antiserum. The antigen-antibody complex was firmly fixed by complement. On staining the antigens with fluorescent antibody, adsorption of the labelled antibodies took place. Unspecific conjugated proteins of the fluorescent antiserum were removed by subsequent washing. In the preparation of fluorescent antisera there is justification for the use of fractions of immune sera containing the maximal quantity of antibodies.

1430

Mikhailov, I.F.; Lavrentev, N.I. 1961. Stainability of para-agglutinating strains of Escherichia coli with fluorescent sera of different specificity. *J. Microbiol. Epidemiol. Immunobiol.* 32:1464-1469.

Para-agglutinating strains of E. coli absorbed serum proteins of differing specificity to various degrees. Fluorescent staining reactions were variable, some heterologous conjugates giving staining of E. coli equivalent to that of homologous pathogenic bacteria. This problem must be considered in diagnostic situations.

1440

Mikhailov, I.F.; Li Li. 1958. The possibilities of using fluorescent antisera in the bacteriological diagnosis of organisms of the intestinal group. J. Microbiol. Epidemiol. Immunobiol. 29:1882-1888.

Experiments involving bacteriological diagnosis of the organisms of typhoid and bacillary dysentery, Shigella dysenteriae, using fluorescent antibodies are described. Standard techniques were employed for globulin precipitation and conjugation using fluorescein isocyanate. Fluorescent antibodies were used to stain and compare fluorescence of S. typhosa, Shigella, and E. coli. The following antisera and their globulin fractions agglutinated Staphylococcus albus: anti-S. typhosa at 1:100, anti-S. flexneri at 1:400, and anti-S. shigae at 1:400, but no FA controls or tests using these organisms were reported. E. coli was the only organism used in controls, and this organism was not agglutinated by the antisera. A method for fluorescent staining of microcolonies on membrane filters was outlined. Results from this procedure were described as orientating and required subculture from the membranes for identification. Test results were poor in attempts to specifically stain haptens of S. typhosa and Shigella adsorbed on red cells.

1450

Mirolubova, L.V. 1962. A possibility of detecting typhoid-paratyphoid bacilli in the blood with the aid of luminescent sera. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:3:14-17. In Russian.

An accelerated method for detecting typhoid-paratyphoid bacilli in the blood was elaborated. It consisted of accumulation for up to 18 hours at 37 C of bacteria in the liquid nutritive medium with their subsequent concentration by centrifuging. The precipitate was examined with the aid of luminescent sera on a slide. This procedure took not more than an hour. Experimental investigations have demonstrated a high sensitivity of this method in comparison with the classical bacteriological method of hemoculture isolation.

1460

Mirolubova, L.V.; Dvurechinskaya, G.S. 1962. The use of the fluorescent serological method for the diagnosis of typhoid fever and paratyphoid A and B. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:10:26-30. In Russian.

The authors have shown on clinical material the possibility of using the fluorescent antibodies, both direct and indirect method, for detecting the causative agents of typhoid fever and paratyphoid A and B in blood with a view to accelerating the diagnosis of these infections. Globulin fractions of fluid-agglutinating and adsorbed typhoid and paratyphoid A and B antisera, labeled with fluorescein isocyanate, were used as fluorescent antibodies in the direct method. Rabbit monoreceptor salmonellosis O-sera, receptor II, IV, V, IX, and VI, and donkey antirabbit serum labeled with fluorescein isocyanate were used for the indirect fluorescent serological method of blood examination. A total of 126 persons were examined, including 71 cases of typhoid and paratyphoid A and B. The blood samples were obtained from the patients on admission to the hospital or within the first two days of their hospitalization.

Hemocultures were positive in 25 cases; fluorescent serological examination gave positive results in 41 cases. The latter reaction proved to be negative in 55 patients with various fevers other than typhoid or paratyphoid A and B. Analogous results were seen with the indirect method, carried out with 59 blood samples.

1470

Nelson, J.D.; Whitaker, J.A.; Hempstead, B. 1960. Diagnosis of enteropathogenic E. coli diarrhea by fluorescein-labeled antibodies. *J. Pediatrics* 57:684-686.

The fluorescent antibody test is a rapid and reliable method for the detection of enteropathogenic Escherichia coli in rectal swab specimens. Cross-staining with the other pathogenic and nonpathogenic organisms does not cause difficulty in interpreting the results of fluorescence. Previously administered antibiotics may inhibit cultural growth but not fluorescent staining. The fluorescent antibody test is superior to standard bacteriologic methods in rapidity, economy, simplicity, and in the detection of a greater number of patients with enteropathogenic E. coli infection.

1480

Nelson, J.D.; Whitaker, J.A.; Hempstead, B.; Harris, M. 1961. Epidemiological application of the fluorescent antibody technique: Study of a diarrhea outbreak in a premature nursery. *J. Amer. Med. Ass.* 176:26-30.

When four cases of diarrhea due to enteropathogenic E. coli 0119:B14 appeared in the nursery for premature infants, an epidemiological investigation was undertaken using fluorescent antibody and standard culture methods. By fluorescent antibody staining of rectal smears, it was determined within 3.5 hours that 25 per cent of the infants were colonized with the same pathogenic serotype of E. coli. An asymptomatic carrier of E. coli 0119:B14 was detected among the nursery personnel by FA. Stool cultures failed to reveal the extent of the infection. The pathogen was recovered from only two of the seven infants having positive FA smears after repeated subcultures. Vigorous control measures, including neomycin therapy to all the infants, were adopted, and the epidemic was stopped without the necessity of closing the nursery to new admissions. There were no deaths directly attributed to diarrhea among the nine colonized infants. FA is admirably suited to epidemiological study of diarrheal outbreaks due to enteropathogenic E. coli because of its high degree of specificity, speed, and sensitivity in detecting carriers.

1490

Osipova, I.V. 1962. Experience of using the fluorescent antibodies for accelerated diagnosis of typhoid fever and paratyphoids. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 33:9:77-83. In Russian.

This work was aimed at ascertaining the possibility of using the indirect fluorescent antibody method for accelerated diagnosis of typhoid fever and paratyphoids. Preliminary investigations in a comparative study of the fluorescent antibody method and agglutination reaction on a slide demonstrated certain advantages of the first of these methods. The main investigations were carried out with artificially infected human blood and the blood of typhoid or paratyphoid patients. Rabbit salmonella

monoreceptor sera and fluorescent antirabbit gamma globulin conjugate labeled with DANS were used for investigations. As shown by observations, the fluorescent antibody method for accelerated diagnosis of typhoid and paratyphoid fevers could be regarded as a tentative one. The employment of the fluorescent antibody method accelerated the blood examination results by 12 to 24 hours.

1506

Page, R.H.; Stulberg, C.S. 1961. Fluorescent antibodies in the epidemiologic control of infantile diarrhea. *Bacteriol. Proc.* M86:123.

Fluorescent antibody techniques were used to determine the incidence of cross-infections as well as the incidence of infant and adult carriers. When FA procedures detected organisms in fecal smears, confirmation was obtained by bacteriologic procedures developed for this purpose. From foci of frank diarrhea due to EEC serotypes 026, 055, and 0111, studies of the spread of the organisms throughout involved areas of the hospital were carried out in 11 separate surveys. In all but one instance, cross-infections with the particular serotype were detected in infants who had been admitted to the hospital for other causes. In addition, adult carriers were identified. Follow-up surveys revealed that proper prophylactic procedures prevented widespread dissemination of the enteropathogens. These results will be compared with retrospective studies of similar cross-infections that occurred at a time when early detection by FA procedures was not available, and where undetected cross-infections subsequently developed into infantile diarrhea of epidemic proportions.

1510

Page, R.H.; Stulberg, C.S. 1962. Immunofluorescence in epidemiologic control of E. coli diarrhea. *Amer. J. Dis. Child.* 104:149-156.

Hospital cross-infections originating from infants with acute diarrheal disease due to enteropathogenic E. coli, EEC, and the incidence of EEC in asymptomatic infants as well as adult carriers were studied. Stool specimens were collected from infants hospitalized for E. coli diarrhea, premature infants on admission, infants in whom diarrhea developed after admission, and infant and adult contacts of such patients. EEC was detected by fluorescent antibody and confirmed by culture. From primary sources, 12 cross-infections occurred in an exposed population of 69 infants. The cross-infections were recognized at a time when the infants were asymptomatic and the enteropathogens were present in exceedingly small numbers. When there was E. coli diarrhea in the nurseries involved, asymptomatic incidents ranged from 6 to 11 per cent. When there was no E. coli diarrhea in the hospital, there was no incidence in two nurseries studied, and in a third only one of 31 infants was infected. Fluorescent antibody was the only means by which early cross-infections could be detected, permitting effective control of hospital-acquired infections.

1520

Petuely, F.; Lindner, G. 1958. A simple, quick method of recognizing pathogenic *E. coli* in fecal smears with the aid of fluorescent antibodies labeled with 1-dimethylamino-naphthalene-5-sulphonic acid as a fluorescent dye. Arch. Kinderheilk. 158:248-252. In German.

Fluorescent antibodies were used to identify dyspepsiacoli in stools. Of 100 stools examined, positive results were found in five cases. The usual method used as control gave a positive result with only three of these five stools. The method allows the clinician to diagnose dyspepsiacoli immediately after the child's admittance and in this way avoid spread of a house infection.

1530

Poetschke, G.; Uehleke, H.; Killisch, L. 1957. Investigations with fluorescein-labeled antibodies: II. Serological properties of the L-phase of proteus. Z. Immunitätsforsch. 114:4:406-415. In German.

The bacterial and the stable as well as the unstable L-phase of a strain of *Proteus morganii* were examined by means of fluorescein-labeled antibodies prepared against all three growth types. All three growth types were colored by these three sera to the same degree. The generic relationship of the bacterial and L-phase is confirmed by these results. Microscopical examination did not disclose a special localization of antigen within the cells. Absorption experiments suggested that the L-phase contains antigens not present within the bacterial growth phase.

1540

Poetschke, G.; Uehleke, H.; Killisch, L. 1959. Studies with fluorescence-marked antibodies: V. Simultaneous demonstration of several antigens by different colored fluorescent antibodies. Schweiz. Z. Allg. Pathol. Bakteriologie. 22:5:758-765. In German.

The labeling of immune gamma globulins with the sulfochlorides of various fluorescent dyes is described. Sulfo-rhodamine B, a pigment with reddish-yellow fluorescence, proved to be a feasible contrasting agent for hydroxy-pyrene-trisulfonic acid chloride and other green-fluorescent labeling substances. *P. morganii* and *B. cereus* were used to demonstrate that simultaneous specific staining with a mixture of variously labeled antibodies is possible. The potential of the method, as well as its advantages and disadvantages, is discussed.

1550

Reimers, E. 1962. Routine diagnosis of dyspepsiacoli using the fluorescence method. Monatsschr. Kinderheilk. 110:5:300-304. In German.

This report concerns fluorescent microscopic routine studies of 300 infant stools in five dyspepsiacoli groups, 026, 055, 0111, 0119, and 0127. The method is discussed. One hundred and two positive results were found, although dyspepsiacoli was found only 28 times with the usual culture methods. Follow-up studies of 34 positive

fluorescent results showed that we were actually dealing with microscopically determined groups, and why, in many cases, the culture procedure had failed. The fluorescent method proved itself simple, quick, and reliable in our studies. It should be urgently recommended to every child clinic.

1560

Riggs, J.I.; Loh, P.C.; Eveland, W.C. 1960. A simple fractionation method for preparation of fluorescein-labeled gamma globulin. *Proc. Soc. Exp. Biol. Med.* 105:655-658.

A relatively simple method for separation of fluorescein isothiocyanate-labeled gamma globulin from serum has been presented. The procedure has been applied to a number of labeled antibacterial and antiviral sera, and the labeled gamma globulin obtained gave definite specific fluorescence when used in the staining procedure, with markedly low background fluorescence.

1570

Riggs, J.L.; Seiwald, R.J.; Burckhalter, J.H.; Downs, C.M.; Metcalf, T.G. 1958. Isothiocyanate compounds as fluorescent labeling agents for immune serum. *Amer. J. Pathol.* 34:1081-1097.

Two fluorescent isothiocyanate dyes have been synthesized for use in labeling antibodies. The advantages of these isothiocyanates over the corresponding isocyanates depend upon their stability in a solid form that allows their storage and use directly when needed. The substitution of a less toxic substance, thiophosgene, for the highly toxic phosgene gas offers added advantages. The dyes were successfully conjugated with various antisera. Specific staining of the following antigens was carried out, using the direct and indirect method, B. anthracis, P. tularensis, R. typhi, and adenovirus RI-67.

1580

Rozhanskiy, I.N.; Galperin, Ya.L.; Dolgov, A.F. 1961. Organization of special training of the medical personnel of units and ships at a sanitary epidemiological laboratory base. *VoennaMed. Zh.* 3:22-25. In Russian.

Courses were given to bacteriological physicians on the use of fluorescent antibodies for the detection of pathogenic microbes. Of primary interest was the detection of microbes in the water by means of fluorescent antibodies.

1590

Rubenstein, H.S.; Fine, J.; Coons, A.H. 1962. The distribution of endotoxin in the dog in lethal endotoxemia as determined by fluorescent antibody. *Federation Proc.* 21:275.

Three dogs were given intravenously a lethal dose of endotoxin, 4 mg per kg; E. coli O111:B4, Westphal chemotype X; Difco, Boivin-Johnson-Landy. One animal was sacrificed 10 minutes after administration of toxin; another, after 90 minutes. The third was allowed to die of endotoxemia 13 hours after toxin. Representative blocks of all

organ systems were quick-frozen. Sections 5 microns thick were cut in a cryostat and acetone-fixed. They were stained by the indirect method: layer 1: rabbit antiserum against E. coli 0111:B4; layer 2: antirabbit gamma globulin, horse, conjugated with fluorescein isothiocyanate. Qualitatively the distribution of endotoxin in the three dogs was identical: Within the cells of the reticuloendothelial system, the Kupffer cells of liver, reticular cells of spleen, septal cells and alveolar phagocytes of lung, sinus lining cells of spleen, lymph node, adrenal, and pituitary; in histiocytes; in circulating and tissue neutrophils; in cells of the adventitia of venules; in capillary endothelium; and, rarely, in endothelium of venules and arterioles.

1600

Sanford, J.P.; Hunter, B.W.; Donaldson, P. 1962. Localization and fate of Escherichia coli in hematogenous pyelonephritis. J. Exp. Med. 116:285-294.

Hematogenous E. coli pyelonephritis was produced in rats. The localization of the organisms and the persistence of bacterial antigens was followed by fluorescent antibody techniques as well as by standard histological and bacteriological methods. Salient sequential features were as follows: Single organisms passed through vessel walls into the renal interstitium and began multiplication and subsequently evoked a leucocytic response. Bacterial multiplication did not occur in glomeruli or renal tubular cells. Bacteria did not appear within renal tubular lumina until microabscesses were well developed in the renal interstitium. Bacteriuria appeared late and represented secondary invasion rather than filtration of organisms. The infection healed spontaneously but, although sterile, the parenchymal scars contained large amounts of residual bacterial antigen. The persistence of bacterial antigen did not result in continuing inflammatory changes or progressive scarring. The persistence of bacterial antigens is postulated to constitute a major antigenic stimulus responsible for active immunity in experimental hematogenous pyelonephritis.

1610

Shaughnessy, H.J.; Lesko, M.; Dorigan, F.; Forster, G.F.; Morrissey, R.A.; Kessner, D.M. 1962. An extensive community outbreak of diarrhea due to enteropathogenic Escherichia coli 0111:B4: II. A comparative study of fluorescent antibody identification and standard bacteriologic methods. Amer. J. Hyg. 76:44-51.

The occurrence of an extensive outbreak of infant gastroenteritis provided an opportunity to compare the results of immunofluorescent identification and standard bacteriologic methods in processing 824 specimens for the presence of enteropathogenic Escherichia coli. In terms of percentage of positive findings, the FA procedure was superior to conventional methods. However, the latter still serves an invaluable purpose in making cultures available for complete antigenic analysis and for antibiotic sensitivity testing. In this regard, all cultures isolated from cases in Illinois during this epidemic were significantly more resistant to antibiotics than the same serologic types isolated in the principal epidemic area. Especially noteworthy was the newly acquired resistance to neomycin sulfate, which had been previously considered to be the drug of choice in treating enteropathogenic E. coli infections.

1620

Silverstein, A.M.; Lveland, W.C.; Marshall, J.D. 1957. Rapid identification of organisms with fluorescent antibodies of contrasting colors. *Bacteriol. Proc.* PH28: 147.

Pairs of antisera, each of which was labeled with either fluorescein or rhodamine, have been used to stain smears of three morphologically identical organisms. Mixed smears of Salmonella typhosa, Proteus vulgaris, and a third gram-negative rod were stained with rhodamine-labeled anti-S. typhosa and fluorescein-labeled anti-P. vulgaris. Examination showed one set of yellow-green fluorescing organisms and a second set of orange fluorescing organisms; a third set of nonfluorescing organisms was demonstrable by darkfield examination. To demonstrate specificity further, the same results were obtained with three types of Diplococcus pneumoniae on mixed smears. The speed and the high degree of specificity of these dual staining reactions suggest their application to the establishment of a schema for the rapid identification of organisms.

1630

Smirnov, V.D. 1961. The use of fluorescent antisera for the bacteriological diagnosis of intestinal infections. *J. Microbiol. Epidemiol. Immunobiol.* 32:1333-1334.

Using both the direct and indirect FA methods, pathogenic Enterobacteriaceae were detected in mixed cultures. Isolated strains could be identified.

1640

Stulberg, C.S.; Cohen, F.; Page, R.H. 1960. Use of fluorescent antibodies to identify a variety of enteropathogenic E. coli serotypes in fecal smears from infants with diarrhea. *Bacteriol. Proc.* M174:141.

This is an attempt to establish whether the combined use of polyvalent and singly labeled antisera would specifically detect and identify the various enteropathogenic serotypes directly in fecal specimens. Fecal smears were prepared from multiple stool specimens taken from 53 infants at various stages of their infections with E. coli serotypes 026:B6, 055:B5, 0111:B4, 0119:B14, or 0127:B8. Globulin fractions were prepared from antisera to the above serotypes and to four others, 086:B7, 0124:B17, 0125:B15, and 0126:B16. After conjugation with fluorescein isothiocyanate, these were combined into two polyvalent pools. The labeled polyvalent antibodies were successfully used to screen the specimens for presence of fluorescent organisms, and the individually labeled antibodies were used to identify the serotype. The usual bacteriologic procedures were carried out in parallel. The fluorescent antibody methods proved to be both sensitive and specific. Few extra-generic reactions were observed, and these did not cause difficulty in the recognition of the specific serotype.

1650

Tanaka, N.; Nishimura, T.; Yamaguchi, H.; Yoshiyuki, T. 1960. Histochemical studies on experimental typhoid by means of fluorescein-labeled antibody: II. Cellular localization of the antigens of Salmonella in the process of the infection of experimental typhoid. Jap. J. Microbiol. 4:35-41.

Cellular localization of antigens of Salmonella enteritidis in mouse tissues after infection with a virulent strain was studied, employing a double-layer method of the fluorescent antiglobulin technique. After a large inoculum, localization of the bacterial antigens was principally the same as observed using killed vaccines, in the Kupffer cells, in the leukocytes around the sinusoids in the liver, and in the mononuclear cells of the red pulp in the spleen. Following a small inoculum they were not readily demonstrated within 48 hours in the liver and spleen. On the 3rd day bacterial antigens appeared in the liver and in the spleen. On the 7th or 8th day, the antigens were concentrated in the Kupffer cells and in the leukocytes that infiltrated around the sinusoids. The amount of the observable antigens decreased after 9 days. Bacteria were not observed in sections stained by conventional histological methods. The staining properties of the bacilli and the difference in the size indicated that the status of the bacilli in the animal was substantially different from that observed in vitro. Typhomas may be formed by the antigen-antibody reaction of Salmonella antigens.

1660

Tanaka, N.; Nishimura, T.; Yoshiyuki, T. 1959. Histochemical studies on experimental typhoid by means of fluorescence-labeled antibody: I. Cellular localization of the antigens of Salmonella enteritidis after injection of killed vaccines. Jap. J. Microbiol. 3:267-274.

The cellular localization of the antigens of Salmonella enteritidis in mouse tissues after intravenous and intraperitoneal injections of killed organisms was investigated, using the double-layer method of FA. Heat-killed organisms and the chrome-vaccine were comparatively studied. In the liver, the antigens of S. enteritidis filled the cytoplasm of Kupffer cells and leukocytes, both monocytes and granulocytes, and infiltrated around the sinusoids and other vessels. Antigens were demonstrated in granulomas in various amounts; intracytoplasmically in epithelioid and other cells. In the spleen, the antigens were localized in the cytoplasm of the large round mononuclear cells, chiefly reticulum cells, of the red pulp. Heat-killed vaccine accumulated in a few layers of cells lining the lymphoid follicles. However, chrome-vaccine was found in cells of the red pulp. The other distribution differences are described. Localization of antigenic particles of killed organisms was markedly different from that of endotoxin. Significance of distribution of the antigens is discussed, particularly the role of antigen and antibody in formation of the granuloma.

1670

Tanaka, N.; Yamaguchi, H.; Nishimura, T.; Yoshiyuki, T. 1960. Histochemical studies on experimental typhoid by means of fluorescein-labeled antibody: III. Demonstration of gamma globulin or antibody in the typhoid granuloma. Jap. J. Microbiol. 4:433-449.

Homologous gamma globulin has been successfully demonstrated in the typhoid granuloma of mouse liver and spleen following injections of live organisms or killed vaccine of a virulent strain of Salmonella enteritidis. It is established by the antigen inhibition test that gamma globulin in the typhoid granuloma is the specific antibody. The Kupffer cells lack gamma globulin or antibody. The observations indicate that antibody may not be transferred from other antibody-synthesizing cells but may be produced by these macrophages. In the spleen, another prominent locus of gamma globulin is observed in cells of the plasma cell series, including Russell bodies. Smaller amounts of gamma globulin are detected in the lymphocytes. Gamma globulin is found chiefly in the cytoplasm. A special type of macrophage produces antibody in particular circumstances; this does not mean that all the cells of the macrophage system produce antibody. This study presents one of the cellular bases of antibody formation against bacterial antigens, which differs from the mechanism against protein antigens.

1680

Thomson, B.M.; Boris, M.; Montague, T.S.; Cherry, W.B. 1962. The incidence of enteropathogenic Escherichia coli among children hospitalized with diarrhea as determined by fluorescent antibody and isolation procedures. Bacteriol. Proc. M145:98.

Certain serotypes of Escherichia coli are a major cause of diarrhea in children under two years of age. This study indicates frequent occurrence of EEC among such children admitted with diarrhea. Approximately 90 per cent of these patients were screened for the presence of EEC. Fecal specimens were collected and smears prepared and treated with pools of fluorescent antibodies for nine enteropathogenic serogroups of E. coli. All FA-positive specimens were cultured, and isolates were subjected to complete serological studies. Forty-seven per cent of the children were positive by FA for one or more EEC; 36 per cent of these were positive by culture for the same serogroup. According to the distribution of age and the multiple serotypes isolated, the infections appear to be of a communal origin. The high incidence of E. coli diarrheas admitted to the pediatric wards presents a potential risk of a hospital outbreak.

1690

Thomson, B.M.; Cherry, W.B. 1960. Detection of enteropathogenic Escherichia coli in dacron and cotton swabs by fluorescent antibody and cultural techniques. Bacteriol. Proc. M175:142.

Fecal specimens preserved in buffered glycerol saline have been found unsatisfactory for fluorescent antibody examination until the glycerin is removed. Frozen specimens are satisfactory for both FA and cultural studies. A series of experiments were undertaken to evaluate the efficiency of dacron and cotton swabs for the detection of enteropathogenic E. coli in fecal specimens. Duplicate fecal swabs were prepared from stool specimens found positive for enteropathogenic E. coli by culture, from broth

cultures of E. coli, and from normal stools seeded with E. coli. One set of swabs was frozen immediately, the other set was allowed to dry at room temperature and remain at that temperature. Sampling was done at various time intervals, and FA and culture results were noted. EEC were recovered more frequently from dried fecal swabs than from frozen fecal swabs. There was little difference between dacron and cotton swabs. Either type was more efficient when pretreated with phosphate buffer. EEC was identified, both by FA and culture, from fecal swabs dried for as long as 8 weeks.

1700

Thomason, B.M.; Cherry, W.B.; Davis, B.R.; Pomales-Lebron, A. 1961. Rapid presumptive identification of enteropathogenic Escherichia coli in fecal smears by means of fluorescent antibody: 1. Preparation and testing of reagents. Bull. WHO 25:137-152.

In outbreaks of infantile diarrhea caused by enteropathogenic Escherichia coli, rapid detection of the etiological agent is essential for specific and adequate therapy. The development of fluorescein-labeled antibodies specific for nine serogroups of E. coli and the testing of pure cultures of various Enterobacteriaceae as well as fecal specimens from cases of diarrhea are reported. These data show excellent correlation between the fluorescent antibody technique and conventional serological methods for identifying enteropathogenic types of E. coli. The immunofluorescent technique is more rapid than the usual bacteriological and serological methods in that smears from fecal specimens can be stained directly with the labeled antibodies. Details of the procedures for carrying out the technique are given.

1710

Thomason, B.M.; Cherry, W.B.; Edwards, P.R. 1958. Fluorescent antibody examination of fecal smears for enteric pathogens. Bacterial. Proc. PH8:134.

This report describes an attempt to use fluorescein-labeled antibodies to identify enteric pathogens directly from stool specimens. Labeled antibodies used were Salmonella polyvalent, S. typhosa O, and Vibrio comma. Use of fluorescent antibodies on mixed flora of stools is fraught with problems of cross-reactions and equivocal results. Stool specimens were also processed for enteric pathogens by culture. Twenty-five specimens were from typhoid carriers. Smears were made either directly or from centrifuged filtrates and stained. In almost every specimen, including those from healthy individuals, there were apparently specific brightly fluorescing objects resembling bacteria. Lactose-positive organisms were isolated that were agglutinated by both the Salmonella polyvalent and by one or more grouping sera. Study of the flora of normal stools and the relationship of naturally occurring bacteria to enteric pathogens is needed. Fluorescent antibodies provide a useful tool for this purpose. Without thorough knowledge of the various cross-reactions, caution is urged in interpreting results obtained by direct staining of fecal smears with fluorescent antibody.

1720

Thomason, B.M.; Cherry, W.B.; Edwards, P.R. 1959. Staining bacterial smears with fluorescent antibody: VI. Identification of salmonellae in fecal specimens. *J. Bacteriol.* 77:478-486.

The potential value of employing fluorescent antibody solutions for the direct identification of salmonellae in fecal smears has been investigated. Serological cross-reactivity was a serious problem. No method has been found to resolve this difficulty, although the problem may be less important in certain specialized areas of application. Incomplete antibody may influence the interpretation of fluorescent antibody tests to a significant degree. These tests constitute an effective tool for measuring the prevalence of this antibody. The importance of analyzing preimmunization sera to prevent misleading interpretations of fluorescent antibody tests is stressed. Variations in types of staining reactions obtained are pointed out and their significance is discussed. The value of fluorescent antibody as a tool for the study of cellular architecture is emphasized.

1730

Thomason, B.M.; Cherry, W.B.; Ewing, W.H. 1959. Rapid identification of serotypes of *Escherichia coli* with polyvalent antibody globulins labeled with fluorescein. *Bacteriol. Proc.* M108:90.

Since there are at least nine serotypes of *E. coli* that may be etiologic agents of infantile diarrhea in this country, a polyvalent reagent might permit more rapid screening of specimens for the presence of these organisms. The globulin fractions of antisera to nine *E. coli* serotypes were labeled with fluorescein isothiocyanate. Two pools were prepared containing antibodies representing four and five serotypes. These were tested against antigens representing each constituent type and cross-reacted with 53 additional antigens. Smears of fecal specimens were tested with the two fluorescent antibody pools. All specimens containing fluorescing bacteria were tested with the labeled globulins for the various serotypes represented in the pools. Fluorescent antibody pools were as sensitive for detection of the 0127:B8 cells as were the type-specific antibodies. The specificity was such that only weak reactions were obtained with a few heterologous cultures and an occasional fecal smear from a culturally negative patient. FA results were largely those predicted from knowledge of serologic relationships.

1740

Thomason, B.M.; Cherry, W.B.; Moody, M.D. 1957. Antigenic analysis of *Salmonella typhosa* by means of fluorescent antibody and agglutination reactions. *Bacteriol. Proc.* M128:102.

Monospecific O, Vi, and H antisera of *S. typhosa* were prepared in rabbits. An antiserum containing all three antigen components also was prepared. Crude globulin fractions of the sera were conjugated with fluorescein isocyanate. When smears of the typhoid organism were exposed to the labeled antiglobulins, the three classes of antigens found in the cells could be stained specifically both collectively and individually. Six strains of *S. typhosa*, one Vi containing paracolon, one *S. virginia*,

one *S. derby*, five *S. typhimurium*, and five *S. bredeney* strains were examined by agglutination and fluorescent antibody reactions. Specificity of the staining established by inhibition and absorption tests with fluorescent antibody agreed closely with that of agglutination tests for all serotypes. No evidence was obtained to suggest that the application of globulins containing antibodies for three classes of antigens either interfered with or enhanced specific staining of these antigens, either singly or in combination. The tests substantiated the difference in somatic, envelope, and flagellar antigens of *S. typhosa*.

1750

Thomason, B.M.; Cherry, W.B.; Moody, M.D. 1957. Staining bacterial smears with fluorescent antibody: III. Antigenic analysis of *Salmonella typhosa* by means of fluorescent antibody and agglutination reactions. *J. Bacteriol.* 74:525-532.

The serological specificity of globulins labeled with fluorescein isocyanate has been studied with *Salmonella typhosa*. The three classes of antigenic components found in cells of this species could be stained specifically both collectively and individually with appropriate labeled sera applied to bacterial smears fixed on glass slides. The specificity of staining established by inhibition and adsorption tests with fluorescent antibody agreed closely with that of agglutination tests for all serotypes. No evidence was obtained to suggest that the application of a polyvalent globulin solution containing one or more types of heterologous antibody interfered in any way with specific staining of homologous antigen.

1760

Thomason, B.M.; Cherry, W.B.; Pomales-Lebron, A. 1961. Rapid presumptive identification of enteropathogenic *Escherichia coli* in fecal smears by means of fluorescent antibody: 2. Use of various types of swabs for collection and preservation of fecal specimens. *Bull. WHO* 25:153-158.

Fecal specimens that cannot be cultured immediately for enteric pathogens are usually collected in buffered glycerol saline solution for preservation and transport to the laboratory. Specimens collected in this are not suitable for fluorescent antibody studies because the glycerol prevents fixation of fecal smears. Transport of specimens frozen with solid carbon dioxide is a satisfactory but expensive method of preservation. The purpose of the study reported in this paper was transporting fecal specimens to be examined for enteropathogenic *E. coli*. Both cotton and dacron fibers were used and freezing and desiccation were investigated as methods of preservation. The results showed that freezing of fecal swabs was contraindicated, whereas drying at room temperature enhanced the recovery of enteropathogenic *E. coli*. Cotton and dacron swabs gave equivalent results regardless of whether the organisms were detected by culture or by immunofluorescence.

1770

Tomov, A.; Spasov, S.; Benvenisti, I. 1962. Application of fluorescent antibodies in the quick diagnosis of dyspeptic coli bacteria. *Suvremenna Med.* 13:8:25-32. In Bulgarian.

Results using DANS-labeled antibodies are reported. The conjugates were claimed to have high specificity and were useful in diagnostic applications.

1780

Truant, J.P. 1959. Staining smears of Salmonella with fluorescent antibody. *Bacteriol. Proc.* M107:90.

Salmonella groups A, B, and D antisera and their globulin fractions were conjugated with fluorescein isocyanate and isothiocyanate. Precautions were taken to obtain comparable preparations by performing agglutination tests and electrophoretic separations, and by obtaining their emission spectra. Direct and indirect staining procedures have been used through these studies. Brilliant fluorescence was obtained in most instances when the Salmonella species were sealed and stained with specific group conjugates. It should be emphasized that labeled globulins usually stained the organisms more intensely. The direct staining procedure was superior to the indirect method. Certain differences were noted in the intensity of fluorescence on the cell surfaces, probably because of such factors as availability and concentration of labeled antibody and antigen as well as because of the method of mechanical and physical-chemical reaction during the staining and rinsing procedures. Tests for group specificity showed relatively weak cross-reactions between group B conjugates and group A antigens. Little difficulty was encountered in staining Salmonella in blood and spinal fluid. Fluorescent organisms and objects were more frequently observed in screening normal fecal specimens.

1790

Whitaker, J.A.; Page, R.H.; Stulberg, C.S.; Zuelzer, W.W. 1958. Rapid identification of enteropathogenic Escherichia coli 0127:B8 by the fluorescent antibody technique. *Amer. J. Dis. Child.* 95:1-8.

This paper describes the application of the fluorescent antibody technique to the identification of pathogenic serotypes of E. coli. Stored frozen material from an epidemic of enteropathogenic E. coli 0127:B8 diarrhea in 1954, extensively studied by conventional methods, permitted a thorough evaluation of the specificity and sensitivity of the fluorescent antibody method. The technique has a high degree of specificity and is more sensitive and more rapid than identification by culture and specific agglutination. It is possible to identify organisms that have lost the power of reproduction or are present in such small numbers that they escape detection in the mixed stool flora. The fluorescent method can be used directly with specimens of stool or rectal swabs. A restudy provides new insight into therapeutic effects and epidemiologic patterns.

1800

White, R.G. 1954. Observations on the formation and nature of Russell bodies. Brit. J. Exp. Pathol. 35:365-367.

Cytoplasmic bodies appear in mature plasma cells in the spleen of rabbits and mice following repeated intravenous injection of various bacillary vaccines. These bodies resemble Russell bodies in morphology and staining reactions. Russell bodies appear at 3 days and are at a maximum in 7 to 9 days in mice following recall antigenic stimulus. Intracytoplasmic crystals developing in a mature plasma cell have the same staining reactions as Russell bodies. The surface of Russell bodies produced in mouse spleen by P. vulgaris injections stained intensely when treated with anti-P. vulgaris serum. Therefore, specific antibody is a major component of Russell bodies.

1810

Wood, C.; White, R.G. 1956. Experimental glomerulo-nephritis produced in mice by subcutaneous injections of heat-killed Proteus mirabilis. Brit. J. Exp. Pathol. 37: 49-59.

Acute or subacute diffuse glomerulo-nephritis was produced in about 50 per cent of mice by repeated subcutaneous injections of heat-killed P. mirabilis, continued for 2 to 11 weeks. Using fluorescein-labeled antibody, antigenic material from P. mirabilis was found to be localized in the glomerular cells. After a single subcutaneous dose, antigen was shown to persist for at least 3 weeks in glomeruli. It is suggested that the localization of bacterial antigen in glomerular cells may be causally related to the production of nephritis and that this localization site is related to the type of glomerulo-nephritis produced in mice, which resembled Ellis type 1 nephritis in man.

1820

Zinovieva, I.S.; Shpagina, M.K. 1962. The use of fluorescent serum for accelerated diagnosis of dysentery. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:12:112-115. In Russian.

Fluorescent serum was tested for identifying Flexner dysentery bacilli directly in the patient's feces. Two hundred and two patients, mainly with dysentery and dyspepsia, were examined in parallel by conventional bacteriological and fluorescent-serological methods. Flexner dysentery bacilli were detected bacteriologically in 21 cases and by the fluorescent-serological method in 42 cases. Positive results of both methods of the investigation coincided in 19 cases. No nonspecific cellular fluorescence was observed in the preparations.

VII. LACTOBACILLACEAE

1830

Anonymous. 1959. Fluorescent antibody identification of group A Streptococci from throat swabs. U.S. Department of Health, Education, and Welfare, Communicable Disease Center, Atlanta, Georgia.

The procedure for group A Streptococcus identification from throat swabs is presented. Sources of materials and services are included.

1840

Cole, R.M.; Hahn, J.J. 1962. Cell-wall replication in Streptococcus pyogenes: Immunofluorescent methods applied during growth show that new wall is formed equatorially. *Science* 135:722-724.

Group A streptococci of several serological types were grown in fluorescein-labeled homologous-type or group-specific globulins, thereby labeling the antigen-containing cell walls. Specific precipitation or inhibition of the labeled antibody, followed by continued incubation and examination at intervals by ultraviolet, phase, and dark-field microscopy, showed that new cell wall was then nonfluorescent. These nonfluorescent portions were differentiated by a reverse technique of culture in unlabeled globulin, followed by antibody precipitation, further growth, and fluorescent antibody staining. This technique of differential labeling of cell wall has permitted following, for the first time in a living system, the fate of cell wall formed at different times. The results suggest that cell wall synthesis in actively growing cultures usually occurs simultaneously at two sites or more per coccus, each site representing stages in successive divisions, and that cell-wall growth in Streptococcus pyogenes is not by diffuse intercalation with old wall, but is initiated at, and extends both peripherally and centripetally from, the coccal equator.

1850

Coons, A.H.; Creech, H.J.; Jones, R.N. 1941. Immunological properties of an antibody containing a fluorescent group. *Proc. Soc. Exp. Biol. Med.* 47:200-202.

A beta-anthrlyl-carbamido derivative of antipneumococcus III rabbit antibody retains the original immunological properties while rendering type III pneumococci specifically fluorescent in ultraviolet light.

1860

Coons, A.H.; Creech, H.J.; Jones, R.N.; Berliner, E. 1942. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J. Immunol.* 45: 159-170.

Impure fluorescein isocyanate has been prepared and successfully conjugated through the ureide linkage to pneumococcal 3 antibody. This fluorescein-carbamido-pneumococcal 3 antibody solution possessed the same agglutinin titer as the original serum in terms

of protein content, and rendered pneumococcus 3 fluorescent in the ultraviolet light. The tissues of mice heavily infected with pneumococcus 3 could be specifically stained in localized areas with this antibody conjugate, and it is proved that this staining is immunologically specific. Incomplete data are presented that suggest that the antigen-antibody reaction is reversible.

1870

Emmart, E.W.; Cole, R.M.; May, E.L.; Longley, J.B. 1958. Studies on streptococcal hyaluronidase and antihyaluronidase: II. The localization of sites of absorption of streptococcal hyaluronidase - group C - with fluorescent antibody. J. Histochem. Cytochem. 6:161-173.

Antihyaluronidase globulin, isolated from the sera of rabbits immunized with streptococcal hyaluronidase, is capable of completely inhibiting in vitro the hydrolytic action of the enzyme upon hyaluronic acid. Conjugation of the antihyaluronidase globulin fractions to fluorescein isocyanate does not affect the antibody. Using FA, streptococcal hyaluronidase has been identified in tissues of mice following the injection. Treatment of normal tissues with fluorescent antihyaluronidase globulin solution, treatment of experimental tissues with fluorescent normal globulin, and blocking of staining action by preliminary absorption of the tissue with unconjugated antibody were controls. Streptococcal hyaluronidase injected intravenously is widely distributed in tissues but is absorbed and retained in selected areas and cells. Selection of specific cells within any given tissue suggests that the basis for absorption of enzyme may be the functional or the physiological state of the cell as well as the histogenesis..

1880

Emmart, E.W.; Turner, W.A., Jr. 1960. Studies on streptococcal hyaluronidase and antihyaluronidase: III. The production and cellular localization of hyaluronidase following streptococcal infection. J. Histochem. Cytochem. 8:273-283.

Streptococcal hyaluronidase and the bacterium Streptococcus hemolyticus, group C, strain 7, have been identified within the same tissue section by tagging the former with its specific antibody coupled to yellow-green fluorescein and the latter with group C antisera coupled to acid rhodamine B. The methods used suggest a means of studying the adsorption and activity of other antigenic enzymes and toxins elaborated during the course of infection.

1890

Fox, E.N. 1962. Measurement of streptococcal antigen synthesis with fluorescent antibody. Proc. Soc. Exp. Biol. Med. 109:577-579.

A technique is described for fluorometric estimation of the somatic M antigen of group A streptococci. The amount of fluorescein-conjugated antibody adsorbed and eluted from cells was a function of the amount of antigen present during the process of antigenic protein synthesis.

1900

Friou, G.J. 1957. Application of the fluorescent antibody technic to the immunology of the group A Streptococcus. Clin. Res. 5:22.

The Coons technique for visual localization of antigen-antibody in tissues with fluorescein-conjugated antisera has been used to investigate localization of streptococcal antigens in mice. Antistreptococcus sera were prepared by immunizing rabbits with various preparations of group A streptococci. Soluble antigens were extracted from streptococcal cells disrupted by sonic oscillation. When injected intravenously there was localization in the glomerulus for a period of hours. Certain antistreptococcus antisera localized in various tissues of normal mice. This localization is a specific property of these antistreptococcus rabbit sera. It suggests that an antibody is present for an antigen in mouse tissues. This type of localization of fluorescent antisera, and that due to specific localization of injected soluble antigen, were studied.

1910

Hahn, J.J.; Cole, R.M. 1961. Improvement and dynamics of the long-chain test for type-specific streptococcal antibody. Bacteriol. Proc. M60:142.

The long-chain reaction is a useful test for detection of type-specific antibodies to Group A streptococci. By using the statistical frequency distributions of chain-length classes instead of averaging cocci per chain, the test has been made easier, more rapid, more sensitive, and better controlled statistically. The long-chain response increases and decreases with incubation time in a parabolic fashion. Time, appearance, and height of the maximum response depend on antibody concentration and, inversely, on size of initial streptococcal inoculum. The peak response is low and early, and its duration above a given level of significance is short, when antibody is low; with high antibody and the same inoculum size, the peak is higher, later, and of long duration. Increasing inoculum size has the effect of immediately decreasing free antibody. Testing by this size-class frequency method is reproducible and allows ready titration of sera by dilution. The presence of specific homologous antibody, even when fluorescein-labeled, does not decrease streptococcal growth. In long-chain tests in fluorescent antibody, addition of excess homologous antigen extract did not result in redistribution of antibody from the chains. Whether chaining or dechaining is the normal state is unknown. Dechaining could be ascribed to continued growth in the absence of a chaining factor.

1920

Halperen, S.; Donaldson, P.; Sulkin, S.E. 1957. Differentiation of streptococci in mixed bacterial populations by use of fluorescent antibody. Texas Rep. Biol. Med. 15:63-64.

Preliminary experiments were designed to adapt FA to rapid clinical laboratory identification of beta hemolytic streptococci in upper respiratory tract secretions and other specimens. Each of the artificially contaminating organisms was mixed with type 18, group A Streptococcus and stained with fluorescent antibody. The organisms used for these mixtures included Klebsiella pneumoniae, Neisseria sicca, Micrococcus

pyogenes, Diplococcus pneumoniae, and Hemophilus pertussis, as well as streptococci of groups B, C, D, and G. A rabbit anti-type 18, group A Streptococcus serum and a Lederle anti-group A serum were used. Examination of the same fields under ultraviolet illumination and darkfield showed staining of the organisms for which antisera were specific and cross-reactions only with group C streptococci. Five other types of group A streptococci gave similar intensities of staining. Interfacial precipitin tests during the process of fluorescein conjugation demonstrated twofold loss in titer. Paper electrophoresis gave evidence that some albumin and beta globulin remained with the gamma globulin after ammonium sulfate precipitation, but only gamma globulin was in the conjugate.

1930

Halperen, S.; Donaldson, P.; Sulkin, S.E. 1958. Identification of streptococci in bacterial mixtures and clinical specimens with fluorescent antibody. *J. Bacteriol.* 76:223-224.

A number of bacterial species have already been differentiated in artificial mixtures and clinical specimens with fluorescein-conjugated antibody. The bacteria to be identified are stained specifically by treating a smear with fluorescein-conjugated anti-serum and are recognized as typical yellow-green organisms among blue-gray nonspecific elements by means of microscopic examination with high-intensity, filtered ultraviolet light. Results of the application of this procedure to the diagnosis of acute streptococcal pharyngitis are presented here.

1940

Inhorn, S.L. 1962. Fluorescent antibody methods in the diagnosis of disease. *Wisconsin Med. J.* 61:222,228.

This is a general introductory article. Diagnostic applications are pointed out. The TPFA test and FA identification of Streptococcus are being performed at the Wisconsin State Laboratory of Hygiene. Speculation is made on the future of FA in the hygiene lab.

1950

Kaplan, M.H. 1958. Localization of streptococcal antigens in tissues: I. Histologic distribution and persistence of M protein, types 1, 5, 12, and 19 in the tissues of the mouse. *J. Exp. Med.* 107:341-352.

A method is described for detection of streptococcal antigens in tissues using indirect immunofluorescence. This has been applied to histologic distribution in the mouse of M protein, types 1, 5, 12, and 19. Histologic localization of these M proteins was similar, and their rates of disappearance from the tissues were comparable. Major sites of deposition were endocardium and adjacent subendocardium of the heart, alveolar walls of the lung, glomerular tufts of the kidney, and reticuloendothelial cells of liver, spleen, lymph nodes, and adrenal gland. M protein was distributed in considerably lesser concentration in capillary endothelium and connective tissue sites in myocardium, kidney, skin, and gastrointestinal tract. Traces were also present in adrenal cortical cells. It was rarely in cell nuclei. After injection of 0.5 mg of

M protein fraction, the concentration of antigen diminished to undetectable levels in all organ sites by 4 days, except in the renal glomerulus, where traces were visible at 8 days. In mice injected with streptococcal culture intraperitoneally, M protein was detected at sites of focal abscesses in liver and spleen, and on the serous surfaces of these organs.

1960

Kaplan, M.H. 1958. Immunologic studies of heart tissue: I. Production in rabbits of antibodies reactive with an autologous myocardial antigen following immunization with heterologous heart tissue. *J. Immunol.* 80:254-267.

Inoculation of rabbits with whole streptococcal cultures grown in beef heart medium stimulated antibody reactive with normal rabbit heart section, as determined by a modified immunofluorescent technique. The immunofluorescent reactivity of the serum was correlated with complement-fixing antibodies to normal rabbit heart tissue suspension. This antibody response was not related to the streptococcus but to the beef heart medium in which the streptococci were grown. Immunization with beef heart medium alone or with homogenate of beef heart also elicited both types of antibody. Similar results were obtained following immunization of rabbits with homogenates of rat and human heart. These data give evidence of a cross-reacting myocardial antigen in the sarcoplasm of the several mammalian species tested.

1970

Kaplan, M.H. 1959. Autoantibodies to heart tissue in the sera of certain patients with rheumatic fever. *Federation Proc.* 18:2263:576.

Indirect FA technique using sera of patients with rheumatic fever frequently will exhibit immunofluorescent staining of tissue sections of normal human heart. This staining reaction is due to the presence in such sera of either of two reactive gamma globulins. The first has been found only in the sera of certain patients with rheumatic heart disease and gives a staining pattern with characteristic juxta-myofibrillar and subsarcolemmal distribution. This staining capacity may be specifically absorbed with heart tissue homogenate; alcoholic and saline extracts of heart have been ineffective. In the second type of reaction, given by rheumatic and certain other pathologic sera, the staining of myofibers is similar but not identical to that described above. Further, this staining capacity is associated with complement-fixing and flocculating activity of the sera with alcoholic extracts of normal heart. One stimulus for the production of the first type of autoantibody may be cardiac surgery itself. The pattern of sarcoplasmic staining given by this autoantibody is similar to the pattern of distribution of deposited gamma globulin with myofibers of some rheumatic specimens. This observation suggests that in these patients autoantibody to a myofiber constituent may participate in the pathogenesis of rheumatic heart disease.

1980

Kaplan, M.H.; Coons, A.H.; Deane, H.W. 1950. Localization of antigen in tissue cells: III. Cellular distribution of pneumococcal polysaccharides types II and III in the mouse. *J. Exp. Med.* 91:15-30.

The cytological distribution of the pneumococcal polysaccharides, types II and III, was followed in the tissues of the mouse. The most constant and striking concentrations of these polysaccharides were found in the cells of the reticuloendothelial system, the ordinary capillary endothelium, and fibroblasts throughout the body. In addition, polysaccharide was detected in monocytes and lymphocytes, hepatic cells, cardiac and smooth-muscle cells, uterine epithelium, and in steroid-forming cells in the adrenal cortex, testis, and ovary. The persistence of polysaccharide, type III, in the tissues was studied. It remained for at least 75 days in the macrophages of lymphoid organs, the Kupffer cells of the liver, and the interstitial macrophages in the myocardium, the lung septal cells, the capillary endothelium, and the renal glomerulus. After a single injection, it persisted for at least 6 months in many areas. The smallest dose that produced detectable amounts in any cells 24 hours after injection was 0.03 mg. Polysaccharide distribution is compared with that of acid vital dyes and suspensoids, and the significance of its fixation in relation to its antigenicity and possible toxicity in mice is discussed.

1990

Kaplan, M.H.; Meyeserian, M. 1962. An immunological cross-reaction between group A streptococcal cells and human heart tissue. *Lancet* 1:706-710.

Widespread deposits of bound gamma globulin were observed in the ventricular muscle of a patient who died of acute rheumatic fever with severe cardiac failure. The possible occurrence of an antigen in group A streptococci immunologically cross-reactive with heart tissue was investigated. Antisera prepared in rabbits against group A streptococcal cell walls exhibited immunological reaction with human heart tissue by immunofluorescence and complement fixation. The reactant in human heart was distributed in cardiac myofibrils and in smooth-muscle elements of arteries, arterioles, and endocardium. Serological reaction was abolished by absorption of antisera with streptococcal cells or cell walls, but not by cell protoplasm or protoplast membranes. Cross-reactive antigen was associated with M protein, but not with A carbohydrate. Immunization of rabbits with such active fractions elicited antibodies reactive with human heart. In rheumatic fever an autoimmune reaction to a constituent of myofiber and smooth muscle may be induced by exposure to cross-reactive antigen of group A streptococci.

2000

Kaplan, M.H.; Meyeserian, M. 1962. An immunologic cross-reaction between group A streptococcal cells and a constituent of human heart tissue. *Arth. Rheum.* 5:1114.

Antisera prepared in rabbits against group A streptococcal cells and cell walls and isolated subfractions therefrom exhibited an immunologic reaction with human heart tissue as determined by the immunofluorescent technique, complement-fixation, and antiglobulin consumption. The culture medium was controlled to exclude extraneous

antigens. The A-5 Streptococcus strain originated from an acute rheumatic fever case. In FA studies the reactant in human heart was distributed in cardiac myofibers and in smooth muscle of arteries, arterioles, and endocardium. Serologic reaction was abolished by absorption of antisera with streptococcal cell walls. Various other immune and normal sera gave negative results. Antigen was found in HCl extracts of whole cells and cell walls. Other preparations destroyed the antigen. Salt fractionation and gradient elution studies are described. Extracts were employed successfully as immunizing antigens. In rheumatic fever, an autoimmune reaction to a constituent resident in cardiac myofibers and smooth muscle may be induced by exposure to a cross-reactive antigen of group A streptococci. This interpretation is supported by our previous finding of bound gamma globulin in both myofibers and vessel walls in rheumatic hearts.

2010

Kaplan, M.H.; Vaughan, J.H. 1959. Reaction of rheumatoid sera with synovial tissue as revealed by fluorescent antibody studies. *Arth. Rheum.* 2:356-358.

Immunofluorescent studies revealed that gamma globulin may become fixed in synovial tissue during nonspecific inflammation. This gamma globulin is also reactive with rheumatoid factor. Both rheumatoid factor and reactant gamma globulin may occur bound in the tissue.

2020

Kohler, W.; Wagner, M. 1962. Typing of group A hemolytic streptococci: IV. The presence of R 28 antigen in streptococci groups B, C, and G, and its assay with fluorescein-marked antibodies. *Z. Immunitätsforsch.* 123:200-207. In German.

Among 56 strains of hemolytic streptococci agglutinating with type 28 antiserum of group A, either exclusively or in combination with the sera of types 8, 9, 13, 18, or group G, the group determination with Fuller extracts revealed 6 strains of group B, 3 strains of group C, and 5 strains of group G. The agglutination is attributed to the R 28 antigen. The partially missing reaction with the agglutinating group sera C and G can be explained by the blocking of the C-polysaccharide by the R 28 antigen. The suspicion arose that other groups than group A may be present when the smears treated with fluorescent antibodies of group A do not react. The specific fluorescence could be demonstrated in a part of the B, C, and G strains when a serum containing R 28 antibodies was used for staining. The absence of fluorescence is explained by the minimal formation of R 28 antigen, which is demonstrable only with agglutination. The findings make it necessary to examine all strains reacting with type 28 antiserum in regard to their group. The examination is not necessary when the agglutination occurs within the reactions with the T antigen groups 4/24... For the production of fluorescent antisera of group A, no sera containing R 28 or T 2 antibodies must be used because otherwise false positive reactions with the groups B, C, or G may occur.

2030

Miller, F. 1961. Renal localization and persistence of type 12 streptococcal M protein. *Proc. Soc. Exp. Biol. Med.* 108:539-543.

The findings of Kaplan with regard to the renal localization of type 12 streptococcal M protein have been confirmed and extended. It has been shown that this property is not the result of denaturation during preparation. The significance of these findings in regard to hypotheses concerning the pathogenesis of acute nephritis is briefly discussed.

2040

Moody, M.D. 1961. Fluorescent antibody identification of group A streptococci from throat swabs. U.S. Department of Health, Education, and Welfare, Communicable Disease Center Release.

The FA method gives rapid and reliable identification of group A streptococci. Success depends on adherence to tested procedures, reagent standardization, and proper use of equipment.

2050

Moody, M.D.; Baker, C.N.; Pittman, B. 1962. Identification of group A streptococci by a simplified fluorescence inhibition test. *Bacteriol. Proc.* M80:83.

Methods presently used for identifying group A streptococci by fluorescent antibody techniques have limitations. Reagents exhibit cross-reactions or require inhibitory media. An improved fluorescence inhibition uses easily prepared reagents, and use of inhibitory media is obviated. Strains of groups A, C, and G streptococci and *S. aureus* each were stained in a single step with predetermined dilutions of group C or A antistreptococcus serum. Group A fluorescent globulins were used before and after absorption with cells of group C streptococci. Blocking of cross-reactions was effected with group C antiserum in high dilutions. No blocking of FA reactions of group A strains was observed. The negative control reagent, consisting of group A fluorescent globulin and group A antiserum, blocked staining of all strains. Results obtained with group A fluorescent globulin before and after absorption with group C cells were the same. Such a test offers the possibility of using more economically prepared reagents that will result in rapid laboratory identification of group A streptococci in a few hours. Fluorescent inhibition tests are applicable to 2- to 24-hour broth cultures or to blood agar plate cultures despite bacterial contamination.

2060

Moody, M.D.; Ellis, E.C.; Updyke, E.L. 1958. Grouping streptococci in dried smears with fluorescent antibody. *Bacteriol. Proc.* PH11:135-136.

Specific fluorescent antibody can be produced that stains specifically all group A streptococcal strains tested and strains of no other streptococcal group. The reagent was prepared by adsorbing group A fluorescent antibody with group C organisms. This removed the cross-staining reactions with group A cells. The reaction was performed

on dried smears prepared from cultures and directly from throat swabs obtained from patients with acute streptococcal throat infections. More limited studies showed that specific fluorescent antibody reagents also could be prepared for streptococcal groups B, C, D, F, and G. Advantages of the procedure include, first, rapidity, inasmuch as a definitive grouping reaction was obtained on smears made directly from swabs or very young broth cultures; second, economy of sera, since the fluorescent antibody solution could be diluted but not for the precipitin test; economy of materials and media, since isolation of pure cultures was unnecessary; and lower personnel costs, since time-consuming steps required for preparing reagents for the precipitin tests were eliminated.

2070

Moody, M.D.; Ellis, E.C.; Updyke, E.L. 1958. Staining bacterial smears with fluorescent antibody: IV. Grouping streptococci with fluorescent antibody. *J. Bacteriol.* 75:553-560.

A specific fluorescein-labeled antibody reagent was prepared that stained all group A streptococcal strains tested and strains of no other streptococcal group. The reagent was prepared by adsorbing group A fluorescent antibody with group C organisms. The adsorption removed the cross-staining reaction with group C organisms but did not affect its affinity for group A cells. The reaction was performed on dried smears prepared from cultures and directly from throat swabs obtained from patients with acute streptococcal throat infections. More limited studies showed that specific fluorescent antibody reagents also could be prepared for streptococcal groups B, C, D, F, and G. The advantages of rapidity and economy offered by the use of specific streptococcal fluorescent antibody for grouping streptococci were discussed.

2080

Movat, H.Z. 1960. Pathology and pathogenesis of the diffuse collagen diseases: III. Pathogenesis of the collagen diseases. *Can. Med. Ass. J.* 85:797-803.

Speaking of the collagen diseases in general, it seems reasonable to conclude, first, that fibrinoid, the characteristic change in these diseases, is not an intrinsic alteration of connective tissue, but the result of exudation, precipitation, and inspissation of plasma proteins; secondly, that there is considerable evidence - morphological, immunological, and experimental - that the lesions of the diffuse collagen diseases are related to an immune mechanism. Only in rheumatic fever is there conclusive evidence that an exogenous agent, namely the beta hemolytic Streptococcus, is implicated in the development of the disease. In some cases of polyarteritis nodosa, exogenous agents such as sulphonamide, iodine, or penicillin have been identified. In the other conditions of the group no exogenous agent is known, and the currently accepted view is that they represent an altered state of reactivity of the tissues, which involves perhaps the production of antibodies against endogenous tissue components.

2090

Peeples, W.J.; Spielman, D.W.; Moody, M.D. 1961. Field application of the fluorescent antibody technics for Streptococcus identification. Amer. J. Public Health 51:920-921.

This field trial conclusively proves that the fluorescent antibody identification of Streptococcus group A is a practical, exact, and useful technique, widely used by Montgomery County physicians. The procedure can be used effectively by local health department laboratories with recommended equipment and training, and can provide physicians with a speedy, accurate laboratory diagnosis.

2100

Peeples, W.J.; Spielman, D.W.; Moody, M.D. 1961. Field application of fluorescent antibody technique for identification of group A Streptococcus. Public Health Rep. 76:651-654.

In a study of the field application of the fluorescent antibody technique for identification of group A beta hemolytic Streptococcus, the technique was found to be 95 per cent or better in agreement with the conventional 2- to 4-hour broth culture method. The fluorescent antibody technique is a rapid, accurate, practical laboratory procedure that can be used by any local or state public health agency or private laboratory.

2110

Petueiy, F.; Lindner, G. 1959. A method of analyzing the intestinal flora by means of fluorescent antibodies. Schweiz. Z. Allg. Pathol. Bakteriologie. 22:5:747-751. In German.

The intestinal flora of infants was studied. This was an attempt to overcome the problem of no growth from 98 to 99 per cent of organisms present in the stool. Gram stains, darkfield, and FA were used. Technical problems are discussed. Infants possessed a nearly pure flora of Lactobacillus bifidus, not demonstrable in the stools of nursing mothers.

2120

Pittman, B.; Moody, M.D. 1950. Staining of Staphylococcus aureus with fluorescein-labeled globulin from nonimmunized and Streptococcus-immunized animals. Bacteriol. Proc. M170:140.

Heat-fixed smears of viable cell suspensions stained with labeled group A antistreptococcus or normal rabbit serum globulin were examined by FA. Fourteen S. aureus and four coagulase-negative Staphylococcus epidermidis albus strains were tested. S. aureus, but not S. epidermidis, stained brilliantly with high dilutions of different preparations of group A antistreptococcus and normal rabbit globulin. Agglutinins for S. aureus were demonstrated in titers from 1:40 to 1:2560 in serum of nonimmunized and group A streptococcus-immunized rabbits. Rare low-grade reactions occurred with S. epidermidis. Antibody to S. aureus was removed from conjugates by adsorption with S.

aureus strains but not with S. epidermidis. More than 1800 throat swab smears were examined by FA, using group A streptococcus and normal rabbit globulin conjugates. S. aureus cross-reaction was reported in only four specimens. Because this cross-reaction is apparently insignificant in field studies, and because specific streptococcal titer is occasionally reduced by the adsorption, group A streptococcus FA reagents are not routinely adsorbed with staphylococci.

2130

Rauch, H.C.; Rantz, L.A. 1962. Immunofluorescent identification of group A streptococci in direct throat smears. Bacteriol. Proc. M75:81.

During the winter of 1960-1961, 767 pharyngeal swabs were obtained from patients both with and without upper respiratory disease. Swabs were cultured and hemolytic streptococci were grouped serologically. Smears prepared directly from these same swabs were stained, using commercial sera, by indirect FA. Serological groupings of 160 beta hemolytic streptococci were: A, 121; B, 1; C, 11; G, 5; 22 could not be group-categorized. Among cultures presenting few hemolytic streptococci on the original plate, 63 per cent were group A, but 90 per cent of cultures presenting large numbers of streptococci were group A. Immunofluorescent examination of original throat smears was positive in 53 per cent of specimens in the former and in 93 per cent of the latter group. Replicative slides examined with absorbed antiserum revealed fluorescent streptococci in 4 per cent of specimens in the group where large numbers of streptococci were originally cultured. Cross-reactions seldom occurred. However, 24 smears from culturally negative swabs revealed fluorescent cocci. Routine immunofluorescent examination of direct throat swabs may be applied in the clinical laboratory.

2140

Redys, J.J.; Ross, M.R.; Borman, E.K. 1960. Inhibition of common antigen fluorescence in grouping streptococci by the fluorescent antibody method. J. Bacteriol. 80:823-829.

Cross-reactions for groups C and G in fluorescein-globulin conjugates prepared from group A streptococcal antisera could not be eliminated by dilution. Adsorption of conjugates reduced intensity of heterologous fluorescent antibody staining but did not completely eliminate crosses with all strains encountered. Adsorption, furthermore, materially reduced homologous activity and, therefore, did not present a completely satisfactory solution. A technique for inhibition of common antigen fluorescence has been used. The application of this technique to smears permitted prompt reporting of group A streptococci. Parallel platings on sheep blood agar have revealed that these primary broth cultures fail only when streptococci are present in comparatively small numbers and hence may have been inhibited in the broth. The technique for inhibition of common antigen fluorescence depends upon treatment of heat-fixed smears with crude group C antiserum in predetermined dilution prior to fluorescent antibody staining.

1250

Redys, J.J.; Ross, M.R.; Borman, E.K. 1960. Inhibition of common antigen fluorescence in grouping streptococci by the fluorescent antibody method. *Bacteriol. Proc.* M168:139.

Difficulties experienced in routine grouping by the fluorescent antibody method of beta hemolytic streptococci isolated from man have led to the successful application of a sequential inhibition technique for the suppression of fluorescence due to antigens common to two or more groups. This technique for the inhibition of common antigen fluorescence, ICAF, has been applied to a large number of isolates and has been found to yield preparations that can be interpreted rapidly and accurately, even by persons relatively inexperienced in fluorescence microscopy. The ICAF technique facilitates the routine screening of isolates for group A and is useful in differentiating between other groups, notably C and G. It eliminates the necessity for heterogeneous adsorption of conjugated antibody. Data suggest that the ICAF technique yields results at least as reliable as those obtained by the more laborious precipitin test.

2160

Schmidt, W.C. 1952. Group A Streptococcus polysaccharide: Studies on its preparation, chemical composition, and cellular localization after intravenous injection into mice. *J. Exp. Med.* 95:105-119.

A method was developed for the extraction of the group A Streptococcus polysaccharide employing pepsin digestion of ground streptococcal cells. This method did not result in the isolation of polysaccharide with chemical and physical-chemical properties different from those exhibited by preparations extracted with hot formamide. Studies of the chemical composition of this polysaccharide showed it to be composed chiefly of rhamnose and glucosamine monosaccharide units in the approximate ratio of five moles of rhamnose to two moles of glucosamine. The fate of the polysaccharide after intravenous injection into mice was studied by the fluorescent antibody technique. It was found to be rapidly eliminated by the kidney. The presence of the polysaccharide in the renal tubular epithelial cells during the excretory phase was the only evidence of its cellular localization that could be detected under the conditions of these experiments.

2170

Seegal, B.C.; Hsu, K.C.; Fiaschi, E.; Andres, G.A. 1959. The technic of fluorescent antibodies applied to the study of the pathogenesis of human nephritis. *Russ. Fisiopathol. Clin. Terap.* 31:523-535.

The fluorescent antibody technique has been applied to 22 biopsy specimens obtained from patients in different phases of kidney disease to test for the presence of streptococcal antigens and native human gamma globulins in the kidney. The same test has been performed on renal tissues of two normal subjects and one case of pyelonephritis. With antihuman gamma globulin there were 9 positives in 15 cases. With anti-H. Streptococcus serum there were 5 positives in 22 cases. Four of these were positive also with antihuman gamma globulin. In three of these patients there was high serum anti-streptolysin-O titer. Gamma globulin and streptococcal antigen were found mainly in

the glomeruli. Therefore, it may be possible that in these structures streptococcal antigens and native gamma globulins, which might function as antistreptococcal antibody, were interacting.

2180

Sercarz, E.; Coons, A.H. 1959. Specific inhibition of antibody formation during immunological paralysis and unresponsiveness. *Nature* 184:1080-1082.

Using bovine serum albumin and pneumococcal polysaccharide as antigens, FA was used to study immunologic paralysis in mice. Smaller immunizing doses resulted in greater numbers of antibody-containing cells.

2190

Streamers, C.W.; Williams, P.M.; Wang, W.L.L.; Johnson, R.S.; McGuire, C.D.; Abelow, I.J.; Glaser, R.J. 1960. Evaluation of bacitracin disk and fluorescent antibody techniques in identifying group A beta hemolytic streptococci. *Amer. J. Dis. Child.* 100:552-553.

In a comparison of the two methods using 226 strains of group A streptococci, the methods were found to be almost identical in sensitivity. Strains were selected by the Lancefield method.

2200

Streamers, C.W.; Williams, P.M.; Wang, W.L.L.; Johnson, R.S.; McGuire, C.D.; Abelow, I.J.; Glaser, R.J. 1962. Identification of group A streptococci: Bacitracin disk and fluorescent antibody techniques compared with the Lancefield precipitin method. *Amer. J. Dis. Child.* 104:157-160.

Seven hundred and seventy-six strains of beta hemolytic streptococci, isolated from patients with or without respiratory disease, or from household contacts, were examined by the Lancefield precipitin technique and by the bacitracin disk method. In addition, approximately two-thirds of the strains were also studied by the fluorescent antibody method. With both of the latter techniques, correct identification of group A streptococci was obtained in more than 90 per cent when compared with the results by the Lancefield method. The occurrence of false negatives was rare. The use of bacitracin disks containing 0.02 unit of the antibiotic affords a simple, practical method for identifying group A streptococci and one that is readily adaptable to use in routine clinical laboratories and in office practice.

2210

Suzuki, S.; Furukawa, N. 1962. The application of fluorescent antibody technique in the field of pediatrics. *Shonika* 3:327-333. In Japanese.

This review article discusses the various FA techniques - direct, indirect, and complement staining. It also describes how to prepare specimens, staining methods, and equipment for fluorescence microscopy. Clinical application of FA technique and diagnosis are considered in influenza, streptococcal infection, pertussis, diphtheria, and viral infections.

2220

Wagner, M.B. 1959. Acute cardiac effects of intravenous antigens. *Federation Proc.* 18:2017:512.

Results of previous work involving introduction of antigens and following pathologic cardiac effects are reviewed briefly. This study concerns streptococcal antigens and the use of FA for antigen localization.

2230

Warfield, M.A.; Page, R.H.; Zuelzer, W.W.; Stulberg, C.S. 1961. Immunofluorescence in diagnostic bacteriology: II. Identification of group A streptococci in throat smears. *Amer. J. Dis. Child.* 101:160-163.

A total of 500 throat swab specimens from cases of acute streptococcal infections, treated cases, and nonstreptococcal acute upper respiratory infections were examined by fluorescent antibody and cultural procedures. Correlation between the two procedures was obtained in 67 of these specimens. In an additional 29 cases, the presence of fluorescent organisms in throat smears was correlated with clinical evidence of streptococcal infections, but in these group A streptococci were not isolated. Twelve of these had received effective antibiotic therapy. It was shown that organisms rendered nonviable by antibiotics retained their original fluorescent staining characteristics. Cross-reactions with heterologous groups of streptococci and other bacteria in throat smears were effectively eliminated by adsorption with group C streptococci. It is concluded that staining of throat smears with fluorescent antibodies offers a sensitive, reliable, and rapid method for the early identification of group A streptococci.

2240

Warfield, M.A.; Zuelzer, W.W.; Stulberg, C.S.; Surfitz, O. 1960. Rapid identification of group A beta hemolytic streptococci by the fluorescent antibody technique. *Amer. J. Dis. Child.* 100:642-643.

The use of the FA technique for rapid, specific identification of group A streptococci from clinical specimens is reported. Technical difficulties and their solutions are mentioned, and the authors conclude that the FA method is highly specific and more sensitive than the more conventional cultural methods.

2250

Yarashus, D.A.; Siegel, A.C. 1960. Evaluation of rapid diagnosis of group A streptococci by fluorescent antibody technics. *Circulation* 22:2:835.

The conventional precipitin and FA broth enrichment methods of grouping beta hemolytic streptococci were compared for accuracy and practicality of application in a routine bacteriological laboratory. Immediate fluorescent staining of a smear from a direct throat swab failed to correlate well with the conventional grouping method. Three hundred and seventy-six cultures were obtained; 132 were positive for group A streptococci by the precipitin method. In all but 14, or 10 per cent, of these cultures,

FA gave positive confirmatory results. Fifteen strains were nongroup A by the conventional method. Five of these were positive FA. There were 229 cultures negative or beta hemolytic streptococci, and 23, or 10 per cent, of these revealed positive FA reactions. Problems in technique include a time interval of 4 hours to process broth cultures for fluorescent surveys and fatigue of the individual surveying the fluorescent fields. Ten per cent false positive and 10 per cent false negative FA reactions were encountered. Whether these represent nonviable organisms or non-hemolytic group A streptococci is not yet determined.

2260

Zelenkova, L. 1962. Application of the Coons technique of fluorescent antibodies for rapid diagnosis of streptococcal infections. *Cesk. Epidemiol. Mikrobiol. Immunol.* 11:3:145-149. In Czech.

FA was used for streptococcus diagnosis from 333 nose and throat swabs. Cultures were also made. FA was fast, 3 to 7 hours, and more sensitive, 5.5 per cent more positive for throat swabs and 13.5 per cent for nasal swabs, than were standard culture procedures.

VIII. MICROCOCCACEAE

2270

Blobel, H.; Berman, D.T. 1960. Serologic studies of purified staphylococcal coagulase with special reference to fluorescence microscopy. J. Immunol. 85:244-249.

Rhodamine isothiocyanate-labeled antibody prepared against electrophoretically purified coagulase was employed after absorption with a coagulase-negative strain of Staphylococcus, to differentiate between coagulase-positive and coagulase-negative staphylococci. By application of this reagent, a close association between coagulase production and the staining reaction was observed, but absolute specificity could not be established. Some relationship between the bacteriophage group of staphylococcal strains and the antigenic specificity of free coagulase produced by the corresponding strains was observed in cross-inhibition experiments. Double-diffusion precipitin analysis revealed heterogeneity of the antiserum prepared against highly purified coagulase.

2280

Blobel, H.; Berman, D.T. 1961. Further studies on the in vivo activity of staphylocoagulase. J. Infect. Dis. 108:63-67.

Intravenous injection of coagulase preparations into rabbits resulted in a marked decrease of blood plasma proteins, particularly fibrinogen. Almost complete fibrinogen depletion was observed in animals inoculated with a lethal dose of the enzyme. The same exposure did not affect a rabbit previously hyperimmunized against coagulase. Homogeneous intravascular coagula in dilated capillaries and venules of the lungs and an alveolar edema were the outstanding histopathological changes in rabbits exposed to a lethal dose of coagulase. The presence of the enzyme in the precipitated protein material was demonstrated by the use of fluorescein-labeled anticoagulase.

2290

Carter, C.H. 1959. Staining of coagulase-positive staphylococci with fluorescent antisera. J. Bacteriol. 77:670.

Antisera were prepared against two strains of S. aureus. The antisera were conjugated with fluorescein isothiocyanate according to the method described by Riggs. Two hundred and eighteen coagulase-positive cultures isolated from human and animal sources were received and their coagulase activity was retested with dehydrated plasma. Of the 218 cultures, 201 stained with type I antiserum and all 218 stained with type II antiserum. Viable coagulase-positive staphylococci could not be isolated from two samples of cheese implicated epidemiologically in an outbreak typical of staphylococcus food poisoning, but large numbers of fluorescent cocci were demonstrated by direct staining with types I and II antisera. The fluorescent antibody technique, therefore, provides evidence of the presence of potentially enterotoxigenic staphylococci in foods, and is particularly valuable for the examination of foods in which the microorganisms may have been killed by processing.

2300

Cohen, J.O.; Cowart, G.S.; Cherry, W.B. 1961. Antibodies against Staphylococcus aureus in nonimmunized rabbits. J. Bacteriol. 82:110-114.

Antibody against staphylococci was demonstrated in the serum of nonimmunized rabbits from various sources. Demonstration was by agglutination, Ouchterlony agar gel diffusion, and fluorescent antibody tests. Serum from special pathogen-free rabbits could be used for differentiation of certain staphylococcal strains.

2310

Cohen, J.O.; Oeding, P. 1961. Differentiation of strains of staphylococci by means of fluorescent antibodies. Bacteriol. Proc. M87:124.

The staphylococcus classification system of Oeding was used as a basis for the development of fluorescent antibody reagents. Rabbits were immunized for production of factor sera, and such sera were rendered specific by absorption with one or two strains of staphylococci. Slide agglutination reactions with the factor sera were compared with those of the fluorescent antibody reagents. Portions of the various unabsorbed sera were fractionated, labeled with fluorescein isothiocyanate, and then absorbed with the appropriate strains in an effort to produce fluorescent antibody reagents that would behave in a manner similar to the factor sera. The specific conjugates were tested by both agglutination and fluorescent staining procedures. The fluorescent staining results for factors a, c, and k paralleled the agglutination results. Others behaved differently by fluorescent staining and by agglutination. A labeled reagent for factor h was not achieved, although the conjugate prepared for factor h was satisfactory for slide agglutination typing. Evidence was obtained that this was probably due to differential labeling of antibodies present in the globulin solution. The study demonstrated the feasibility of obtaining specific fluorescent antibody reagents for the typing of staphylococci.

2320

Cohen, J.O.; Oeding, P. 1962. Serological typing of staphylococci by means of fluorescent antibodies. J. Bacteriol. 84:735-741.

Fluorescent antibody reagents for identifying seven antigenic factors of staphylococci have been prepared. The fluorescent staining reactions of these reagents were compared with the agglutination reactions with the diagnostic cultures of coagulase-positive staphylococci. Correlation between the two serological tests was almost complete with factors a, b, i, and k. The c fluorescent antibody reagent had a somewhat broader spectrum of activity than the corresponding agglutination serum, whereas the m fluorescent antibody reagent stained fewer strains than were agglutinated in m serum. The fluorescent antibody reagent for h factor stained strains possessing h₁ factor but not strains possessing only h₂ factor. Fluorescent antibody reagents for specific staphylococcal factors did not stain strains of group A streptococci.

2330

deRepentigny, J.; Sonea, S. 1959. Measurements of intensity of primary fluorescence in microbial smears. *Can. J. Microbiol.* 5:563-565.

A method is presented for preparing suspensions and smears of bacterial cultures and for their subsequent observation for primary or natural fluorescence. The equipment for measurement is described in detail. Intensity reading ranges are given for three organisms, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. The results from various control measurements are compared with these. The investigators felt that the apparatus was very accurate and postulate its use for measuring primary fluorescence intensity of groups of microorganisms isolated in a heterogeneous microscopic field.

2340

Gaedeke, R. 1960. Antigen-antibody localization in lung tissue with fluorescent antibody during interstitial pneumonia. *Monatsschr. Kinderheilk.* 108:152-156.

In an attempt to elucidate the etiology of interstitial plasmocellular pneumonia by analysis of heterogenetic antigens in the lungs, various yeast and staphylococcus antisera were conjugated. The lungs of two infants who died of this disease were examined. Results indicated the possibility of a multiplicity of antigens involved in this disease.

IX. MYCOPLASMA (PPLO)

2350

Barile, M.F.; Malizia, W.F.; Riggs, D.B. 1962. Incidence and detection of pleuropneumonia-like organisms in cell cultures by fluorescent antibody and cultural procedures. *J. Bacteriol.* 84:130-136.

A total of 102 tissue-cell cultures from 17 separate laboratories were examined for pleuropneumonia-like organisms, PPLO, by the fluorescent antibody and cultural procedures. PPLO were isolated from 48 of the 49 tissue-cell cultures found positive for PPLO by the fluorescent antibody procedure, and results of the two procedures agreed in 101 of the 102 cases. PPLO were isolated from none of 10 primary-cell cultures prepared from six animal species and from 48 of 92 continuous-cell cultures prepared from eight animal species. Cells grown in media containing antibiotics were more frequently contaminated with PPLO - 72 per cent - than cells grown in antibiotic-free media - 7 per cent. Ninety-one per cent of the cultures from tissue-culture-producing laboratories and 76 per cent of the cultures used for propagation of microorganisms were contaminated with PPLO, although none used for tissue-culture metabolic studies was contaminated. In addition, our findings support the view that PPLO contamination of cell cultures is probably due to bacterial contaminants that revert to L forms in the presence of antibiotics.

2360

Barile, M.F.; Riggs, D.B. 1961. Immunofluorescence of PPLO in tissue cultures. *Bacteriol Proc.* G44:83.

Fluorescein-conjugated antisera prepared against a PPLO isolated from a tissue culture reacted with PPLO strains isolated from tissue cultures but not with five PPLO strains isolated from the human urethra or with 24 different bacterial species. Positive reactions were obtained from impressions of PPLO colonies and from original cell cultures contaminated with PPLO strains. No staining reactions were obtained with primary tissue cells found free of PPLO by cultural methods. FA was used for detection and surveillance of PPLO in tissue cultures. A survey compared results by the fluorescent antibody technique and by cultural methods. These procedures were done independently and information was not exchanged until tests were completed. A total of 85 primary and continuous cell cultures representing 35 different cell types from 16 separate laboratories were examined. Of these, 50, or 59 per cent, were found positive for PPLO. Complete agreement of results was obtained.

2370

Carski, T.R.; Shepard, C.C. 1961. Pleuropneumonia-like mycoplasma infections of tissue culture. *J. Bacteriol.* 81:626-635.

Several media were compared for growth of PPLO infecting cell lines. Tissue culture medium without cells did not support growth. FA staining showed clumped PPLO antigen on the cell membrane. Antibiotic sensitivity tests of the PPLO revealed sensitivity to 2.5 ug per ml of tetracycline. The original source of the PPLO was not determined although several possibilities were investigated.

2380

Chanock, R.M.; Hayflick, L.; Barile, M.F. 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PFLO. Proc. Nat. Acad. Sci. 48:41-44.

Growth of the Eaton agent on an agar medium is described and some of its growth characteristics are outlined. Fluorescent antibody was used for identification and comparison of the agar colonies.

2390

Chanock, R.M.; Mufson, M.A.; Bloom, H.H.; James, W.D.; Fox, H.H.; Kingston, J.R. 1961. Eaton agent pneumonia. J. Amer. Med. Ass. 175:213-220.

In a controlled 6-month study at a Marine recruit base, infection with Eaton agent was detected in 68 per cent of 238 men with atypical pneumonia. Infection occurred significantly less often, only 6 per cent, among recruits without respiratory illness. The agent was recovered in monkey kidney tissue culture from 14 of 17 recruits with serologically positive pneumonia. These findings suggest that the Eaton agent was associated with pneumonia in the recruits. Fluorescent antibody studies suggested that 44 per cent of the recruits were infected at some time during the 3-month training period. The risk of an Eaton pneumonia during training was also high, 1.5 per cent. Only one in 30 infections was manifest as a clinically diagnosed pneumonia. Infection was also associated with febrile respiratory disease in which pneumonia did not occur; however, the majority of infections appeared to be asymptomatic. Eaton agent was not highly communicable, an attribute that favored its persistence in the troops because it did not infect, and thus immunize, large numbers of recruits as they reported in.

2400

Chanock, R.M.; Rifkind, D.; Kravetz, H.M.; Knight, V.; Johnson, K.M. 1961. Respiratory disease in volunteers infected with Eaton agent: A preliminary report. Proc. Nat. Acad. Sci. 47:887-890.

A recently isolated tissue culture strain of Eaton agent had the capacity to infect humans and to stimulate the production of antibody. All individuals who were free of fluorescent-stainable antibody became infected and a majority developed pneumonia, otitis, or a febrile respiratory illness. Although a majority of individuals who had Eaton antibody at the time of inoculation became infected, the presence of such antibody appeared to protect against the occurrence of moderate to severe illness. This antibody-illness relationship strongly suggests that the Eaton agent itself was responsible for initiating the sequence of events that led to pneumonia, otitis, or febrile respiratory disease. These data and previous observations support the specificity of the fluorescent staining reaction in patients with atypical pneumonia. The frequency of otitis in experimental Eaton infection suggests the need for evaluation of this agent in naturally occurring ear disease and that otitis may be a more common manifestation of infection than pneumonia.

2410

Clyde, W.A. 1961. Demonstration of Eaton agent in tissue culture. *Proc. Soc. Exp. Biol. Med.* 107:715-718.

The Eaton agent was localized in secondary monkey kidney cell cultures by means of fluorescent antibody staining employing either human atypical pneumonia convalescent sera or rabbit antiserum. Morphologic study of material stained by the Giemsa method revealed the occurrence of extracellular growths resembling colonies with an average diameter of 10 microns. These findings plus other characteristics of the agent indicated an intimate relationship to organisms of the genus Mycoplasma.

2420

Clyde, W.A.; Denny, F.W. 1962. Studies on Eaton agent in tissue culture. *Amer. J. Dis. Child.* 104:552-559.

Experiments were conducted on the inhibition of growth of Eaton agent by antibiotics and antisera. FA was used to insure result specificity and to determine FA stainability titers of rabbit antisera. Maximum colony counts were obtained at 5 to 8 days and mean generation time was 9 hours. Effects of antibiotics and antisera were studied by adding them to the tissue culture medium prior to inoculation of the cultures. Rabbit antisera and PAP convalescent patient sera inhibited growth, and this correlated with fluorescent-stainable antibody titers. Antibiotic sensitivity was as follows: sensitive to tetracycline and oleandomycin; partially resistant to tetracycline derivatives, streptomycin, and chloramphenicol, resistant to penicillin, bacitracin, and polymyxin B. Neutralizing capacity of patient sera more specifically relates the organism to PAP than have previous studies.

2430

Clyde, W.A.; Denny, F.W.; Dingle, J.H. 1960. Fluorescent-stainable antibodies to the Eaton agent in human primary atypical pneumonia transmission studies. *J. Lab. Clin. Med.* 56:799-800.

Primary atypical pneumonia in two volunteer groups was studied clinically and by cold agglutinin titrations and fluorescent-stainable antibody titrations. The relationship of the disease in volunteers to fluorescent-stainable antibodies against the Eaton virus was demonstrated. This antibody titer rise was of greater specificity than cold agglutinin response and showed even subclinical infections.

2440

Clyde, W.A.; Denny, F.W.; Dingle, J.H. 1961. Fluorescent-stainable antibodies to the Eaton agent in human primary atypical pneumonia transmission studies. *J. Clin. Invest.* 40:1638-1647.

Preserved sera from 70 volunteers participating in two primary atypical pneumonia transmission experiments were examined for fluorescent-stainable antibodies to the Eaton agent. FA responses were associated with cases of primary atypical pneumonia through subclinical infection. Rises of cold hemagglutinins and Streptococcus MG

agglutinins were limited for the most part to patients with atypical pneumonia and were influenced by the severity of the disease. FA results may represent inapparent or mild infection with the Eaton agent, or they may reflect nonspecific factors. Data presented indicate an association of the Eaton agent with disease in the volunteers studied. These results cannot be interpreted as proof of the etiological role of the Eaton agent in primary atypical pneumonia until the exact specificity of the reaction between the agent and fluorescent-stainable antibodies has been established.

2450

Cook, M.K.; Chanock, R.M.; Fox, H.H.; Huebner, R.J.; Buescher, E.L.; Johnson, R. T. 1960. Role of Eaton agent in disease of lower respiratory tract. Brit. Med. J. 1:905-910.

Considerable serological evidence for the association of the Eaton agent and viral pneumonia, with or without cold agglutinins or Streptococcus MG antibody rises, was obtained by use of the indirect fluorescent antibody test. In two instances serological rises to the agent were substantiated by isolation of a strain of the Eaton agent from the sputum of the patient. Antigenic variation and nonspecific reactions could not be demonstrated. Prevalence of fluorescent antibody to the Eaton agent in a general population group suggests that it is a wide-spread and common infection that may occur in all age groups. Infection with the agent may occur in any month of the year.

2460

Donald, H.B.; Liu, C. 1959. Cytological studies of chick embryo cells infected with the virus of primary atypical pneumonia. Virology 9:20-29.

Elementary bodies have been found by electron microscopy in the non-ciliated epithelial cells of the chick embryo bronchus infected with the virus of primary atypical pneumonia. Infected bronchi were selected by immunofluorescence reactions, employing convalescent human serum and fluorescein-labeled antihuman gamma globulin rabbit serum. The cytoplasm of the infected cells also contained numerous multilaminated structures, but there was little cell damage otherwise evident, nor was there any demonstrable involvement of the nuclei or the cell walls.

2470

Eaton, M.D.; Farnham, A.E.; Levinthal, J.D.; Scala, A.R. 1962. Cytopathic effect of the atypical pneumonia organism in cultures of human tissue. J. Bacteriol. 84: 1330-1337.

The cytopathic effect of 3 strains of atypical pneumonia agent was studied in human amnion and human embryonic lung continuous cell cultures. FA was used to demonstrate clumps of organisms in the cells. Cytopathic effects were apparently associated with intracellular growth.

2480

Eaton, M.D.; Liu, C. 1957. Studies on sensitivity to streptomycin of the atypical pneumonia agent. *J. Bacteriol.* 74:784-787.

Two strains of the primary atypical pneumonia agent were tested for sensitivity to inactivation by streptomycin in vitro and for response to this antibiotic in experimental infections in chick embryos and cotton rats. A recently isolated strain, F. H. 1955, showed significantly greater sensitivity to streptomycin than did strain Mac, which was obtained from human pneumonic lung in 1944. The significance of these findings in relation to the nature of the agent is discussed. Fluorescent antibody technique was used to detect atypical pneumonia agents in tissue cells.

2490

Liu, C. 1957. Studies on primary atypical pneumonia: I. Localization, isolation, and cultivation of a virus in chick embryos. *J. Exp. Med.* 106:455-466.

By means of fluorescein-labelled antibody, the primary atypical pneumonia virus was found to multiply exclusively in the cytoplasm of the epithelial cells lining the bronchioles and air sacs of developing chick embryos. When 13-day-old embryos were inoculated intra-amniotically and incubated at 35 C for 5 days or longer, more than 90 per cent of the inoculated embryos became infected. Between 1954 and 1956, seven strains of PAP virus were isolated from sputums or nasopharyngeal washings in patients during the acute stage of the PAP infection. One strain of virus was isolated from the frozen lung of a patient who died in Boston in 1943. All eight recently isolated strains and the Mac strain isolated by Eaton et al. in California in 1944 were antigenically closely related if not identical. PAP virus is not related antigenically to agents of psittacosis, Q fever, adenovirus types 1 to 6, influenza A or B, or pneumonia virus of mice.

2500

Liu, C. 1961. Studies on primary atypical pneumonia: III. A factor in normal serum which enhances the reaction between PAP virus and convalescent serum. *J. Exp. Med.* 113:111-123.

A factor present in fresh normal human or guinea pig serum enhances the primary atypical pneumonia antibody titers in serological reaction with its homologous virus as tested by indirect fluorescent antibody staining. This factor is not properdin. It has many properties similar to complement but may not be identical with complement. The addition of this enhancement factor to sera of humans convalescent from primary atypical pneumonia has made the serological test more sensitive without affecting its specificity.

2510

Liu, C.; Eaton, M.D. 1955. Study and isolation of primary atypical pneumonia virus in chick embryos by means of fluorescein-labelled antibody. *Bacteriol. Proc.* M2:61.

The Mac strain of primary atypical pneumonia virus, propagated in chick embryos for 10 years, was used. Using human PAP convalescent sera and antihuman gamma globulin prepared in rabbits, PAP virus was localized in the cytoplasm of bronchial epithelial cells of infected chick embryos. The amniotic sac of 10-day chick embryos, incubated further for 7 days at 35 C, was used. Thirty to 40 per cent were positive. Thirteen-day-old embryos inoculated and incubated for 5 days were nearly 100 per cent positive. Fluorescence was noted in tissue and passages. Confirmation was by clinical infection of cotton rats.

2520

Liu, C.; Eaton, M.D.; Heyl, J.T. 1956. Studies on primary atypical pneumonia. *Bull. N.Y. Acad. Med.* 32:170-171.

The indirect fluorescent antibody test was applied to diagnosis of PAP using convalescent sera, antihuman globulin rabbit sera, and viral antigen in infected chick embryo lungs. In patients recovering from proved PAP cases, all sera were positive in 3 weeks after onset. Of 44 Boston area patients, sera were 70 percent positive, and of 27 sera from patients in a New Hampshire boys' school all were positive by the FA test used.

2530

Liu, C.; Eaton, M.D.; Heyl, J.T. 1959. Studies on primary atypical pneumonia: II. Observations concerning the development and immunological characteristics of antibody in patients. *J. Exp. Med.* 109:545-556.

By using the indirect method of fluorescent staining to study the antibody response in patients with primary atypical pneumonia associated with the development of cold agglutinin, it was found that the PAP antibody developed during the 2nd and 3rd week of the illness, persisted more than a year, and is not related to the cold and *Streptococcus* MG agglutinins. The development of the PAP fluorescent-staining antibody paralleled the neutralizing antibody for the PAP virus as tested in cotton rats. The sensitivity of this specific serological test was indicated by the observation that 67 to 92 per cent of the patients in several outbreaks of PAP showed a rise of antibody titer during convalescence. Absorption of the sera with various tissue powders did not affect the PAP antibody detected by this method.

2540

Liu, C.; Heyl, J.T. 1957. Serological study of primary atypical pneumonia. *Federation Proc.* 16:1814:423.

Antibodies to primary atypical pneumonia in humans may be detected by indirect fluorescent antibody test of the bronchial epithelium of 13-day-old chick embryos infected intra-amniotically. A study group of more than 75 human cases demonstrated

an antibody rise in 2 to 3 weeks and persistence for more than one year. These antibodies are distinguishable from other antibodies; 80 per cent of clinically diagnosed cases showed titer rises. Eight strains were isolated that cross-reacted with each other and with the Mac strain of Eaton et al. Negative controls included antisera to mouse pneumonia virus and adenovirus types 1 to 4. Factors indistinguishable from complement enhanced the staining reaction.

2550

Marmion, B.P.; Goodburn, G.M. 1961. Effect of an organic gold salt on Eaton primary atypical pneumonia agent and other observations. *Nature* 189:247-248.

Fluorescent antibodies were used in an attempt to correlate specific antigen with the formation of coccobacilli in chick embryos. Special staining characteristics and organic gold salt sensitivity are reported for the strain of Eaton PAP agent used.

X. NEISSERIACEAE

2560

Brown, L.; Copeloff, M.B.; Peacock, W.L. 1962. Study of gonorrhea in treated and untreated asymptomatic women as determined by fluorescent antibody and culture methods: II. Teenage and young adults confined. Amer. J. Obstet. Gynecol. 84:753-757.

Gonococci can be detected in asymptomatic promiscuous teenagers. The delayed FA technique is capable of detecting gonococcus when gram stain is negative and is comparable to good culture methods. Vagina, cervix, urethra, and anus should all be tested to determine gonococcus presence.

2570

Deacon, W.E. 1961. Fluorescent antibody methods for Neisseria gonorrhoeae identification. Bull. WHO 24:349-355.

The application of FA to N. gonorrhoeae identification promises great value in the epidemiology of gonorrhea. Although much remains unknown of the antigen-antibody reaction, conjugates may be prepared and used for a 16- to 20-hour identification as opposed to 10 days for culture. The delayed fluorescent antibody method is of especial value for the detection of gonorrhea in females.

2580

Deacon, W.E.; Peacock, W.L. 1962. A review of problems concerned with the development of serologic methods of Neisseria gonorrhoeae identification. Amer. J. Public Health 52:855-856.

An investigation of the antigen c mosaic comprising the Neisseria group reveals similarities in common with those of the enteric microorganisms. Shared antigens are common in the somatics and specificity appears to be related to K or capsular-like antigens. Fluorescent-antibody methods, because of a sensitivity far beyond conventional procedures, have demonstrated minor antigenic phase changes in the Neisseria group and the occurrence of normal antibodies in fluorescein-labeled antisera. These problems in relation to Neisseria gonorrhoeae identification were considered and resolved by means of the blocking antibody technique.

2590

Deacon, W.E.; Peacock, W.L., Jr.; Freeman, E.M.; Harris, A. 1959. Identification of Neisseria gonorrhoeae by means of fluorescent antibodies. Proc. Soc. Exp. Biol. Med. 101:322-325.

A species-specific antigen, associated with freshly isolated, inagglutinable gonococcus cultures, is recognized. The new antigen appears to possess characteristics similar to Vi antigen of S. typhi or the K antigens of the Escherichia group, and is fully developed only in freshly isolated cultures or in infectious exudate. Antisera containing GC-K(B) antibodies may be labeled with fluorescein and used as a means of identifying N. gonorrhoeae in smear preparations.

2600

Deacon, W.E.; Peacock, W.L., Jr.; Freeman, E.M.; Harris, A.; Bunch, W.L., Jr. 1960. Fluorescent antibody tests for detection of the gonococcus in women. Public Health Rep. 75:125-129.

Fluorescent antibody methods have been developed for the rapid identification of Neisseria gonorrhoeae in women. A combination of the direct and delayed fluorescent antibody methods was clearly demonstrated as superior to the conventional culture method. The delayed FA method gave a slightly higher yield of positive results in less time than the conventional culture method. The delayed FA method was superior to the direct method alone. The value of vaginal examinations in addition to the customary urethral and cervical tests is indicated.

2610

Harris, A.; Deacon, W.E.; Smith, C.A. 1957. Fluorescent antibody studies at Venereal Disease Research Laboratory. Public Health Lab. 15:8-10.

The nature of the work in this laboratory and the organisms under study are described with further reference to future plans. Descriptions in brief are given of the direct, indirect, and blocking reactions. Indications of the usefulness of each are made. Early findings indicate that fluorescent antibody may be applied both in identification and in more basic antigenic study of the various organisms.

2620

Harris, A.; Deacon, W.E.; Tiedemann, J.; Peacock, W.L., Jr. 1961. Fluorescent antibody method of detecting gonorrhea in asymptomatic females. Public Health Rep. 76:93-96.

Using the delayed fluorescent antibody method, Neisseria gonorrhoeae was detected in 44 of 213 female jail inmates who had no signs or symptoms of N. gonorrhoeae infection. Urethral, cervical, and vaginal sites of these 213 patients were examined and N. gonorrhoeae was found at one or more of these sites in the 44 patients. Examination of all three sites produced more positive findings than did examination of any one site or any combination of two sites. Repeat examinations of 74 of these patients showed that additional positive findings could be obtained by a second examination.

2630

Hunter, D.K.; Ziff, M.; Hess, E.V. 1962. Antibody response in gonococcal arthritis by an immunofluorescent method. Arth. Rheum. 5:649.

The indirect method of immunofluorescence has been utilized in an investigation of the antibody response in a group of patients with gonococcal arthritis and Reiter syndrome and in appropriate control groups. Methods are briefly reviewed. Strong fluorescence was noted with 1:100 dilutions of the acute sera of all of 14 patients with gonococcal arthritis in whom gram-negative diplococci were demonstrated. Titers up to 1:1600 were obtained. Sera of 14 of 21 patients with a clinical but unconfirmed diagnosis of gonococcal arthritis were positive. The sera of only 5 of 23 patients with Reiter syndrome gave positive reaction at this dilution. Seven patients with septic

arthritis due to other organisms, all of 15 patients with acute rheumatic fever, and 14 of 15 patients with active rheumatoid arthritis were not reactive. Positive results were obtained in 5 of 27 acute gonorrhea patients. The method described provides a sensitive means of titrating gonococcal antibody in gonococcal infections as well as permitting epidemiologic study of Reiter syndrome, non-gonococcal urethritis, and related conditions.

2640

Metzger, J.F.; Smith, C.W. 1960. Rapid identification of Neisseria meningitidis by fluorescent antibody technic. *Armed Forces Med. J.* 11:1185-1189.

A method for rapid identification of Neisseria meningitidis by the fluorescent antibody technique, using a polyvalent serum, is presented. Cross-reactions with 20 strains of related Neisseria species were negative, with the exception of a slight cross with one strain of Neisseria gonorrhoeae. Specific staining was not obtained with 107 other organisms tested. The use of this technique in the clinical laboratory is discussed.

2650

Simpson, W.G.; Brown, W.J. 1962. Current status of the diagnosis and management of gonorrhea. *J. Amer. Med. Ass.* 182:63-66.

The incidence of gonorrhea in the U. S. is estimated to be more than 15 million cases per year, but only one-sixth of these are reported to public health authorities. No reliable estimate can be made on the amount of economic loss and degree of mental and physical suffering produced as a result of gonococcal infections. For example, the total extent and cost of the complications of gonorrhea in females cannot be determined. From the viewpoint of medical management, the following laboratory and clinical problems should be given special attention. First, the need for reliable methods of detection and confirmation of the diagnosis of gonorrhea in the female; second, improved methods for determination of adequate therapy in the female patient with gonococcal infection; third, closer consideration and determination of the frequency of treatment failures when penicillin and other antibiotics are given, and their relation to the results of the in vitro test of the gonococcus for susceptibility; and fourth, determination of adequate alternative therapy for patients who either are sensitive to penicillin or do not respond to treatment with this agent.

XI. PSEUDOMONADALES

2660

deRepentigny, J.; Sonea, S. 1958. Microscopic detection of the primary fluorescence of bacteria. *Can. J. Microbiol.* 4:17-23. In French.

Thirty-one bacterial species showed a yellow primary fluorescence, or autofluorescence, that could be observed visually with a commercial fluorescence microscope and recorded on photographic emulsions. The present work emphasizes the importance of this natural property for the study of bacteria, either for taxonomic purposes or as a preliminary step when using fluorochromes. Lack of an easy and reliable technique for detecting primary fluorescence in bacteria is probably the reason that little work has been done in this field. The related subject of macroscopic fluorescence of some Pseudomonas, Azotomonas, and Azotobacter cultures, which is associated with diffusible pigments equally present inside and outside the bacteria, was avoided. The aim was detection of primary fluorescence of the bacterial cell itself.

2670

deRepentigny, J.; Sonea, S. 1958. Measurements of intensity of primary fluorescence in microbial smears. *Can. J. Microbiol.* 5:563-565.

A method is given for preparing suspensions and smears of bacterial cultures and for their subsequent observation for primary or natural fluorescence. The equipment for measurement is described in detail. Intensity reading ranges are given for three organisms, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. The results from various control measurements are compared with these. The investigators felt that the apparatus was very accurate and postulate its use for measuring primary fluorescence intensity of groups of microorganisms isolated in a heterogeneous microscopic field.

2680

Moody, M.D.; Goldman, M.; Thomason, B.M. 1956. Staining bacterial smears with fluorescent antibody: I. General methods for Malleomyces pseudomallei. *J. Bacteriol.* 72:357-361.

Antiserum was prepared in rabbits against Malleomyces pseudomallei and the globulin portion was labeled with fluorescein isocyanate. Thirty-three strains of M. pseudomallei and three strains of Malleomyces mallei showed positive staining reactions with the labeled globulin; 19 strains of other bacterial species gave negative reactions under the same conditions. Staining was effected on air-dried smears prepared from viable or nonviable cells that were fixed in various ways or not fixed. The time required for staining was 10 to 15 minutes, using labeled globulin diluted 1:2 with saline. The immunological character of the staining observed under the conditions employed in this work was demonstrated by inhibition and adsorption techniques. Some implications of these results for the diagnostic laboratory are pointed out.

2690

Moody, M.D.; Thomason, B.M.; Goldman, M. 1956. Staining of Malleomyces pseudomallei with fluorescent antibody. Bacteriol. Proc. M55:81.

The report describes the use of fluorescein-labeled antiglobulin to stain individual cells of Malleomyces pseudomallei in dried smears prepared from cultures and from infected animal tissue. Antiserum showing an agglutinin titer of 1:2550 was prepared in rabbits by injecting them with formalin-killed suspensions of M. pseudomallei. A crude globulin fraction of the serum was conjugated with fluorescein isocyanate. When smears of the homologous organism were exposed to a drop of labeled globulin for about 15 minutes, the bacteria became brightly fluorescent. Thirty-three strains of M. pseudomallei and three strains of M. mallei showed positive reactions after exposure to labeled globulin. Nineteen strains or species of other bacteria remained nonfluorescent after similar exposure. It was possible to detect and identify M. pseudomallei in suspensions containing as few as 220 organisms per ml, both with pure cultures and in mixtures with other bacteria. M. pseudomallei was also identified in smears after being mixed with soil, sprayed in an aerosol, or passed through guinea pigs. In most cases, the elapsed time between preparing the smears and identifying the organisms was less than one hour.

2700

Paton, A.M. 1960. The role of pseudomonas in plant disease. J. Appl. Bacteriol. 23: 526-532.

The role of pseudomonas in plant disease is discussed. FA has been useful in defining the close association of pseudomonas with the plant surface, specifically on washed roots.

2710

Thomason, B.M.; Moody, M.D.; Goldman, M. 1956. Staining bacterial smears with fluorescent antibody: II. Rapid detection of varying numbers of Malleomyces pseudomallei in contaminated materials and infected animals. J. Bacteriol. 72:362-367.

Using fluorescein-labeled anti-Malleomyces pseudomallei globulin, it was possible to identify cells of the homologous species on smears prepared from suspensions containing as few as 220 homologous cells per ml. This occurred when suspensions contained only M. pseudomallei or M. pseudomallei plus other bacterial species in a ratio of as much as 1:10,000,000. In addition, it was possible to identify cells on experimentally seeded soil, on material collected from surfaces previously sprayed with suspensions of the organism, and on tissues of experimentally infected animals.

XII. SPIRILLACEAE

2720

Carter, C.H.; Leise, J.M. 1957. Specific staining of various bacteria with a single fluorescent antirabbit globulin. *Bacteriol. Proc.* PH29:147.

This report describes the use of fluorescent-labeled antirabbit globulin for the detection of bacteria. The antirabbit globulin was prepared by the intravenous injection of methanol-precipitated globulin into a goat. This antirabbit globulin was conjugated with fluorescein isocyanate by the method of Coons and Kaplan. It was possible to stain bacteria in 15 minutes with this fluorescent antirabbit globulin when the bacteria had been previously combined for 10 minutes with homologous rabbit antiserum. With bacteria exposed to heterologous rabbit antisera, no staining occurred. Positive reactions were obtained with Brucella suis, Pasteurella pestis, Pasteurella tularensis, and Vibrio comma, indicating the possibility of specifically staining a number of bacteria with only one fluorescent antiserum. This indirect fluorescent staining method was compared with the direct method using specific fluorescent immune globulin for B. suis and for P. pestis. In each instance results with the direct and indirect methods were comparable. Additional studies showed that small numbers of cells in both pure and mixed cultures could be detected with fluorescent antiserum.

2730

Carter, C.H.; Leise, J.M. 1958. Specific staining of various bacteria with a single fluorescent antiglobulin. *J. Bacteriol.* 76:152-154.

A single fluorescent antiglobulin has been used in conjunction with specific non-fluorescent antiserum to stain specifically Brucella suis, Pasteurella tularensis, Vibrio comma, and Pasteurella pestis. Similar results were obtained in the direct procedure, which required a specific fluorescent antiserum for each of the organisms listed. Thus, the indirect procedure accomplished the same results with one instead of many fluorescent preparations. The indirect procedure was employed with both pure and mixed cultures and with small numbers of cells. It was possible to use successive applications of specific antisera and fluorescent globulin until the organism was identified. Cross-reactions were eliminated by employing low-titered antisera in both the direct and indirect reactions.

2740

Chibrikova, F.V.; Shchurkina, I.I.; Tabakov, P.K.; Mosolova, O.N. 1962. The possibility of using specific fluorescent antibodies for rapid observation of the cholera vibrio in water. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 33:3:9-14. In Russian.

Fluorescent sera were prepared from anticholera agglutinating O-serum. Its globulin fraction was isolated with ammonium sulfate or sodium sulfate and the immune globulins obtained were combined with the fluorescein isocyanate. Investigation demonstrated their usefulness for rapid detection of cholera vibrios in artificially

infected samples of water. With 1 ml of water containing about 1 million vibrios a positive answer was obtained in 1.5 to 2 hours. To detect thousands and tens of thousands of vibrios per ml, it was necessary to concentrate them on a membrane filter with subsequent culturing on solid nutritive medium. The vibrios were detected in the washings from filter after 4 to 5 hours of incubation. A scheme is suggested for rapid examination of water for cholera vibrios with the use of anticholera fluorescent serum and membrane filters.

2750

Finkelstein, R.A.; LaBrec, E.H. 1959. Rapid identification of cholera vibrios with fluorescent antibody. *J. Bacteriol.* 78:886-891.

By combining selective enrichment and fluorescent antibody techniques, a method has been devised that facilitates the rapid recognition and serological identification of small numbers of cholera vibrios, *Vibrio comma*, in human stool suspensions. This procedure minimizes the nonspecific reactions encountered in the direct examination of stool specimens.

2760

Marrack, J. 1934. Nature of antibodies. *Nature* 133:292-293.

Breinl and Haurowitz showed that serum coupled with p-aminobenzenearsinic acid retained some of its specific agglutinating power. The proteins are connected to red azo dyes. Specific sera so treated were tested against their homologous antigens, typhoid bacilli, and cholera vibrios. The specific agglutination reactions were colored pink, but reverse combination of the antigen-antisera mixtures gave no color. The assumption was that the protein dye of the antiserum was specifically taken up by the bacterial antigen.

2770

Mikhailov, I.F.; Li Li. 1959. Detection of *Vibrio comma* on objects of the external environment by means of fluorescent antisera. *J. Microbiol. Epidemiol. Immunobiol.* 30:33-40.

The findings discussed in this paper warrant the assumption that the method of staining of *V. comma* on objects of the external environment with fluorescent antiserum opens additional possibilities for the development of quick methods of bacteriological diagnosis of cholera and deserves to be introduced into practice. Introduction of fluorescent antisera into practice will enable us to simplify and speed up the present schemes of bacteriological identification of several other species of bacteria.

2780

Shantarenko, I.V. 1960. A study of para-agglutination by the fluorescent antibody technique. *J. Microbiol. Epidemiol. Immunobiol.* 31:2033-2036.

FA enables us to discern a specific agglutination reaction from a nonspecific one. The para-agglutination test on slides or in tubes does not always represent an

antigenic relationship even if absorbed type-specific agglutinating Shigella antisera are used. Conclusions concerning the frequency of isolation of so-called atypical strains from objects of the external environment and human feces should be received with due criticism. Horse V. comma antisera O and P. pestis agglutinating antisera used at present are capable of reacting with some strains of E. coli and other bacteria, which must be kept in mind in attempts to detect bacterial contamination in the external environment using these antisera in the indirect fluorescent antibody technique.

2790

Tabakov, P.K.; Chibrikova, E.V.; Shurkina, I.I.; Velner, E.I. 1962. A rapid method of obtaining antibodies labeled with fluorescent stains. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:10:26-30. In Russian.

A simple method for antibody isolation from the agglutinating anticholera and anti-plague sera has been suggested. A new method for purifying the fluorochrome-labeled antibodies from the excessive amount of the stain also has been presented. The employment of the mentioned methods reduces the time necessary for preparing the fluorescent conjugates to 3 or 4 days instead of the 3 weeks required by the classical Coons and Kaplan method; it does not require any special equipment such as refrigerator centrifuges and special rooms. Soviet fluorochrome, or fluorescein isothiocyanate, was tested and the advantages of this preparation over isocyanate fluorescein were demonstrated.

XIII. SPIROCHAETALES

2800

Anselmi, E.; Carlizza, L. 1962. Immunofluorescence test for the diagnosis of syphilis. Policlinico Sez. Prat. 69:43:1529-1534. In Italian.

The specificity and ease of performance of the FTA test are discussed. Advantages to the diagnostic lab are pointed out.

2810

Aru, L.; Scarpa, B. 1962. The FTA test for syphilis diagnosis. Ann. Sclavo 4:5:471. In Italian.

FTA was compared with the VDRL and Wassermann tests, which use cardiolipin and treponemal antigens, with sera of patients in various syphilitic stages. The FTA test was the most sensitive.

2820

Borel, L.J.; Durel, P. 1959. Immunofluorescence applied to the diagnosis of syphilis. Pathol. Biol. 7:2317-2324. In French.

The authors applied the immunofluorescence test to the diagnosis of syphilis. The study covered 41 sera of nonsyphilitic subjects and 153 sera of treated and nontreated syphilitic subjects. The results are compared with those given by the treponema immobilization, or TPI, test and those obtained with cardiolipid tests by Kolmer and Kline. The advantages of the immunofluorescence test are its greater simplicity of execution as compared with the TPI test and its greater specificity as compared with that of the standard tests. In addition, its sensitivity is greater than that of all other tests that have been used to date. The results obtained during this preliminary study allow the authors to consider that the immunofluorescence test is likely to play an important role in syphilography.

2830

Boulanger, P.; Robertson, A. 1961. Fluorescein-labeled antibody technique for the demonstration of Leptospira pomona. Can. J. Comp. Med. Vet. Sci. 25:299-306.

The fluorescein-labeled antibody technique was used in the detection of leptospira in pure culture, in urine, and in kidney tissue. The reactivity of the labeled globulins in the test was somewhat influenced by the method of immunization. Inoculation with live culture yielded a serum with a narrower spectrum of reactivity. Provided that the organisms were present in large numbers in the material under examination, the fluorescent antibody technique was almost as effective as cultural methods in demonstrating leptospira. When the organisms were few, it was difficult to differentiate them with certainty from artifacts.

2840

Censuales, S.; Garofalo, V. 1959. Anti-treponemal antibodies in human syphilis detected with the fluorescence reaction. Riv. Inst. Sieroterap. Ital. 34:3:161-167. In Italian.

The following treponemal tests were carried out on 100 human sera having different serologic patterns: fluorescent treponemal antibody test, FTA; treponemal immobilization test, TPI; Reiter protein complement fixation test; and the cardiolipin complement fixation test. The results obtained show that FTA strictly parallels the behavior of the TPI test. FTA is more simple and rapid and less expensive than the TPI test, but discrimination between positive and negative results proved in some instances to be more delicate as compared with the TPI test. Because of its specificity and sensitivity FTA can be recommended to those laboratories that cannot employ the TPI test. FTA can be advantageously used in the study of the antigenic structure of T. pallidum and of the antigenic relationship between virulent and culturable treponemes.

2850

Coffin, D.L.; Maestrone, G. 1962. Detection of leptospire by fluorescent antibody. Amer. J. Vet. Res. 23:159-164.

The fluorescent antibody procedure applied to the detection of leptospire yielded more successful results than darkfield examination and cultures. Not only were superior results obtained in fresh, uncontaminated material, but positive findings were possible in refrigerated, frozen, and contaminated specimens when other means failed. Organisms could be detected in dried smears stored at room temperature for 1 year and in fluids and tissues preserved in formalin for 1.5 years. Diagnostic value of the method was shown by demonstration of organisms in fresh and preserved urinary sediment, in scrapings from the cut surface of kidneys, and in sections from organs of naturally infected dogs.

2860

Covert, S.V.; Kent, J.F.; Stevens, R.W. 1961. Fluorescent treponemal antibody test using Reiter treponeme. Proc. Soc. Exp. Biol. Med. 106:729-731.

A fluorescence test for treponemal antibody using in vitro cultures of Reiter treponemes compares favorably in sensitivity and specificity for syphilis with one requiring the virulent Nichols strain of T. pallidum, which must be cultured in vivo.

2870

Csermely, E.; Marcheselli, W.; Allegra, F.; Santini, R. 1962. Comparison of the immunofluorescent and complement-fixation reactions for syphilis. Riv. Inst. Sieroterap. Ital. 37:4:503-507. In Italian.

Sera of 300 individuals, 80 healthy donors and 220 syphilitic patients in different stages, were tested by the FTA and complement-fixation tests with cardiolipin and Reiter treponemal antigen. It has been shown that in primary syphilis, positivity of FTA test appeared earlier than in complement-fixation tests. In secondary syphilis

all tests were always in agreement; in late and latent syphilis the FTA test was more frequently positive, 90 per cent, in comparison with complement-fixation tests, 82 per cent. The FTA test and complement-fixation test with Reiter treponemal protein antigen often gave the same result, 203 of 220 cases, or 92 per cent.

2880

Dacres, W.G. 1961. Fluorescein-labeled antibody technique for identification of leptospiral serotypes. *Amer. J. Vet. Res.* 22:570-572.

Fluorescein-labeled antibodies were found to stain Leptospira sp. specifically after the organisms had been fixed with osmic acid vapor, a harsh fixative that destroys many organisms and deteriorates with age.

2890

Deacon, W.E.; Falcone, V.H.; Harris, A. 1957. A fluorescent test for treponemal antibodies. *Proc. Soc. Exp. Biol. Med.* 96:477-480.

A technique for the use of fluorescein-labeled antiglobulin as an indicator of treponemal antibody is described. The effect of rotation as an intensifier of fluorescent antibody reactions is determined. Results obtained with fluorescent antibody tests on syphilitic rabbits are compared with results of other treponemal and nontreponemal tests. Serological discrepancies obtained with fluorescent antibody and several other tests on human sera are presented.

2900

Deacon, W.E.; Freeman, E.M. 1960. Fluorescent treponemal antibody studies. *J. Invest. Dermatol.* 34:249-253.

The FTA test is basically a simple procedure. Problems arising from nonspecificity are most likely concerned with the treponemes, or lipid antigens, and as a result, carefully tested antigens should be selected. That is, each new batch of antigen should be checked for lipid contamination. This may be done by checking with anti-lipid sera. Also, tests should be made for sensitization of the treponemes by rabbit antibodies. Once an antigen has been found satisfactory, it may be stored in several ways, such as refrigeration at 5 C, freezing at -40 C, and by lyophilizing. Fluorescein-labeled antihuman and antirabbit globulins are available commercially. The greatest cost appears to be in purchasing fluorescent equipment. However, with the increasing applications of fluorescent antibody techniques, such an expenditure is justified.

2910

Deacon, W.E.; Freeman, E.M.; Harris, A. 1960. Fluorescent treponemal antibody test: Modification based on quantitation, FTA-200. Proc. Soc. Exp. Biol. Med. 103: 827-829.

A modified FTA test technique designated FTA-200 is described. It was developed by examining well-documented serum specimens from various syphilis categories and a presumed normal group. The FTA-200 test possessed a greater degree of reproducibility than the FTA test when results were compared on duplicate sera.

2920

Deacon, W.E.; Harris, A. 1957. Fluorescent antibody studies in venereal disease. Digest of Proceedings, 8th Annu. Symp. Recent Advances in the Study of Venereal Diseases, Abstr. 8.

The Venereal Disease Research Laboratory has initiated several projects designed to explore the possible application of labeled antibody techniques to chancroid, gonorrhea, and syphilis studies. Although little progress has been shown in the chancroid and gonorrhea projects, work has not been entirely unproductive. Little is known concerning the antigenic behavior of either N. gonorrhoeae or H. ducreyi. Some of the difficulties that have been encountered in the chancroid and gonorrhea studies appear to be antisera of low potency and specificity. Treponema pallidum studies have yielded promising results. Sera obtained from syphilitic rabbits and humans have been labeled with fluorescein and have been found capable of staining T. pallidum directly in smears. Indirect staining has been observed to be sensitive and highly specific in experiments with sera obtained from T. pallidum-infected rabbits and selected human cases of syphilis. Labeled goat antirabbit globulin and goat antihuman globulin have been reliable in these experiments.

2930

Deacon, W.E.; Hunter, E.F. 1962. Treponemal antigens as related to identification and syphilis serology. Proc. Soc. Exp. Biol. Med. 110:352-356.

The antigens of intact T. pallidum, the Reiter treponeme, T. microdentium, and the free-living mud organism, T. zuelzeri, are compared. An antigen appearing to be identical to the Reiter protein antigen was found to be a common or shared component in all of the three treponemes studied. By means of absorption or blocking procedure and employing FA methods, all treponemes could be identified. It is suggested that FA methods can play an important part in future taxonomic studies of treponemes and that the FTA test can be modified so as to increase both sensitivity and specificity.

2940

Edwards, E.A. 1962. Detecting Treponema pallidum in primary lesions by the fluorescent antibody technique. Public Health Rep. 77:427-430.

A method is described for identifying Treponema pallidum on slides from primary syphilitic lesions by the fluorescent antibody technique. Such slides may be mailed to a

base laboratory for examination, thus permitting diagnosis in areas removed from laboratory facilities. Preliminary observations indicate that the method is comparable to the darkfield method of demonstrating T. pallidum.

2950

Fife, E.H.; Bryan, B.M.; Sanders, R.W.; Muschel, L.H. 1961. Evaluation of the fluorescent treponemal antibody test for syphilis. *Amer. J. Clin. Pathol.* 36:105-113.

The FTA test for syphilis was evaluated in tests with 745 sera, and the results were compared with those obtained with the TPI procedure. Test results with the two procedures manifested excellent correlation; agreement was obtained with 667, or 90 per cent, of the specimens. Clinical histories provided criteria for evaluating specificity and sensitivity of the procedures when discrepant results were encountered. Analysis of the findings indicated that the FTA tests were more sensitive and at least as specific as the TPI, and had the added advantage of being relatively simple to perform. The FTA was observed to be a valuable adjunct to the TPI, particularly in tests with sera containing threshold amounts of antibody, and exhibited promise as a standard diagnostic procedure.

2960

Fribourg-Blanc, A.; Niel, G. 1960. Application of immunofluorescence to the serological diagnosis of syphilis. *Bull. Soc. Franc. Derm. Syph.* 67:946-951. In French.

Various techniques for preparation and use of fluorescent antibodies are described. Application of immunofluorescence to the serological diagnosis of syphilis needs to be studied further to learn all the aspects of the evolution of the fluorescent antibody in the course of syphilis, the amount of precision in this technique, the standardization of techniques, the expression of results obtained, and the place that this reaction will take in diagnostic serology.

2970

Fribourg-Blanc, A.; Niel, G. 1962. Serologic diagnosis of syphilis using the fluorescent antibody technique. *Presse Med.* 70:1875-1878. In French.

This article covers many aspects of syphilis diagnosis. Advantages of FA over other techniques are discussed with reference to special situations, i.e., early, secondary, late, and congenital syphilis. Comparisons with other syphilis serological tests are made.

2980

Garson, W. 1959. Recent developments in the laboratory diagnosis of syphilis. *Ann. Intern. Med.* 51:748-758.

Ten syphilis diagnostic tests developed between 1947 and 1958 are reviewed and discussed. These are all treponemal tests. The fluorescent treponemal antibody test is included. The author indicated that this was a rapid test and a good one but was currently limited by the cost of the fluorescent microscopy equipment and the lack of a readily available standardized antigen.

2990

Harris, A.; Bossak, H.M.; Deacon, W.E.; Bunch, W.L., Jr. 1960. Comparison of the fluorescent treponemal antibody test with other tests for syphilis on cerebrospinal fluids. Brit. J. Vener. Dis. 36:178-180.

The FTA test was compared with the TPI, KRP, and VDRL tests in spinal fluids from 369 syphilis clinic patients and 59 pre-anesthesia patients. The results obtained with these four tests are presented and discussed.

3000

Harris, A.; Deacon, W.E.; Smith, C.A. 1957. Fluorescent antibody studies at Venereal Disease Research Laboratory. Public Health Lab. 15:8-10.

The nature of the work in this laboratory and the organisms under study are described with further reference to future plans. Descriptions in brief are given of the direct, indirect, and blocking reactions. Indications of the usefulness of each are made. Early findings indicate that fluorescent antibody may be applied both in identification and more basic antigenic study of the various organisms.

3010

Inhorn, S.L. 1962. Fluorescent antibody methods in the diagnosis of disease. Wisconsin Med. J. 61:222,226.

This is a general introductory article. Diagnostic applications are pointed out. The TPFA test and FA identification of streptococcus are being performed at the Wisconsin State Laboratory of Hygiene. Speculation is made on the future of FA in the hygiene lab.

3020

Jacobs, M.B.; Gerstein, M.J. 1960. Fluorescent antibody staining, p. 245-247. In M.B. Jacobs and M.J. Gerstein, ed., Handbook of microbiology. D. Van Nostrand Co., Princeton, N.J.

This is a brief outline of the FA method, with details given for staining Treponema pallidum.

3030

Kent, J.F.; Covert, S.V.; Reilly, H.W.; Kinch, W.H.; Lawson, W.B. 1962. Evaluation of fluorescent treponemal antibody, RFTA and FTA II, and other tests, RPCF and TPI, for syphilis. Proc. Soc. Exp. Biol. Med. 109:584-589.

Two fluorescent treponemal antibody tests, the Reiter protein complement-fixation test, and the Treponema pallidum immobilization test were compared. The TPFA tests compared favorably with the RPCF test, and TPFA tests enjoyed the advantages of performance simplicity, need for fewer reagents, and use of an in vitro-cultured organism.

3040

Mannucci, E.; Spagnoli, U. 1961. The immunofluorescence reaction for research on syphilis antibodies. Ann. Sclavo 3:1:49-62. In Italian.

The fluorescent test permits results that are comparable with those of the test of Nelson Meyer, the TPI. Its advent simplifies the serodiagnosis of syphilis. The methods of preparation of the required reagents, the technical precautions, and the test reading procedures are described.

3050

Montgomery, C.H.; Suhrland, S.; Know, J.M. 1960. Observation concerning fluorescent treponemal antibody test for syphilis. J. Invest. Dermatol. 35:95-101.

A comparison of the FTA test with the VDRL slide test and RPCF test shows that the FTA becomes reactive earlier in the course of syphilis than either of the other two tests. The FTA either detects an antibody different from that of the TPI test, or it is a more sensitive test than the TPI. Quantitative determinations of FTA antibody were performed by a serum dilution technique. It was found that FTA antibody was detectable in higher titers than was reagin, as measured by the VDRL. The FTA followed much the same pattern as reagin, with the highest titers being found in secondary syphilis. This suggests that the antibody measured is not responsible for the relative immunity observed in late syphilis. The FTA showed a reduction in titer following treatment but was detectable over a more prolonged period than the VDRL.

3060

Moulton, J.E.; Howarth, J.A. 1957. The demonstration of Leptospira canicola in hamster kidneys by means of fluorescent antibody. Cornell Vet. 47:524-532.

L. canicola organisms were demonstrated in hamster kidney sections and in smear preparations by means of fluorescent antibody. The reaction between fluorescent antibody and leptospiral antigen was considered specific because it could be inhibited by pre-treatment with unlabeled leptospiral antiserum and because it did not occur when non-specific fluorescent antibody conjugates were employed.

3070

Niel, G.; Fribourg-Blanc, A. 1962. The modern technique of the immunofluorescence test applied to the diagnosis of syphilis. Ann. Inst. Pasteur Paris 102:616-628. In French.

The authors have studied 5,025 sera and cerebrospinal fluids by qualitative and quantitative immunofluorescence. They describe a simplified technique that can be used in routine practice. The main difficulty of the test, obtaining high titers of adequate anti-gamma globulin, was not eliminated. The method is extremely sensitive and has excellent reproducibility. Its early and prolonged positivity is remarkable. The titers, which range from 50 to 36,000, allow one to follow the evolution of the disease with great accuracy. The specificity for titers as low as 50 and 150 is not always reliable. With the reagents described, the immunofluorescence test adapted to the diagnosis of syphilis can be used in routine practice.

3080

Olansky, S.; McCormick, G.E. 1960. Experiences with the fluorescent treponemal antibody test for syphilis. A.M.A. Arch. Dermatol. 81:59-65.

Comparative results among the VDRL slide test, the KRP test, and the FTA test in 189 human sera are reported. The FTA test apparently measures the same antibody as the TPI test. The FTA test shows great promise as a future aid in the resolution of problems of interpretation of reactions in serologic tests for syphilis.

3090

Pagnes, P. 1962. FTA, TPI, and classical serologic tests: A comparative study in 100 cases. Minerva Dermatol. 37:12:396-398. In Italian.

The subject tests were compared in syphilis cases of various stages. FTA and TPI are more specific. FTA offers the advantage of rapid, early diagnosis. TPI was more specific and quantitative.

3100

Pillot, J.; Borel, L.J. 1961. Study of the antibodies responsible for the immunofluorescent reaction with Treponema pallidum using serum from a patient with syphilis. Compt. Rend. 252:954-956. In French.

The reaction is due to one of two of the specific antibodies of the pathogenic treponemas and to the antiproteins of the group of pathogenic and nonpathogenic treponemas.

3110

Poetschke, G.; Killisch, L. 1959. Studies with fluorescein-labeled antibodies: VI. Fluorescence-serological studies of spirochetes, T. pallidum, Reiter spirochete, Borrelia recurrent, and spirochetes of the mucous membrane. Schweiz. Z. Allg. Pathol. Bakteriол. 22:5:765-770. In German.

Antibodies against human and rabbit T. pallidum and against Reiter spirochete were labeled with fluorescein isocyanate and rhodamine sulfochloride. Specific staining of T. pallidum and Reiter spirochete with both antibodies shows that both spirochetal species have at least one common antigen. B. recurrent and spirochetes from the mucous membrane of the human oral cavity do not share antigens with T. pallidum or Reiter spirochete. Immunization of rabbits with Reiter spirochete causes production of antibodies against cardiolipin. Untreated Reiter spirochetes react differently to agglutinins from anti-Reiter sera and anti-T. pallidum sera when compared with antigen produced from Reiter spirochetes in spirochetal agglutination according to Roemer and Schilipkoeter.

3120

Portnoy, J. 1952. New serologic tests for syphilis. *Electro Lyte* 4:8.

The newer tests for syphilis are discussed with respect to their merits and inherent problems. The fluorescent treponemal antibody test is briefly outlined as a portion of the discussion.

3130

Sheldon, W.H. 1953. Leptospiral antigen demonstrated by the fluorescent antibody technic in human muscle lesions of Leptospira icterohemorrhagiae. *Proc. Soc. Exp. Biol. Med.* 84:165-167.

Leptospiral antigen was demonstrated with the fluorescent antibody technique in the muscle lesions of a patient with L. icterohemorrhagiae infection. This observation indicates that the muscle lesions in Weil's disease are produced by the leptospirae. It has also been shown that the nonspecific staining of human polymorphonuclear leukocytes can be eliminated by absorption of the conjugate with rabbit bone marrow powder.

3140

Siboulet, A.; Fribourg-Blanc, A.; Niel, G. 1962. Practical application of the immunofluorescent technique to the serological diagnosis of syphilis in 100 clinical cases. *Bull. Soc. Franc. Derm. Syph.* 69:892-897. In French.

The FA technique is simple, quick, and yields early results. Although technically simple, it must be of high quality. With a technique such as this that was not sufficiently controlled, the authors were careful to watch for decrease in sensitivity and specificity. The very early results of the FA test in early syphilis always precede those of the TPI and classical cardiolipid reactions. The facts justify increased interest in this technique. It is now considered the technique of choice in diagnosis and therapeutic control of syphilis associated with a single reaction of cardiolipid flocculation. However, the quantitative TPI remains the reaction that specifically resolves any questionable serology.

3150

Silverstein, A.M.; Kent, J.F. 1957. Staining of intact Treponema pallidum with fluorescein-labeled antibodies. *Symp. Venereal Dis. Abstr.* 9:5.

Fluorescent antibody has been used to produce specific immunohistochemical staining of various microorganisms, and the specificity and simplicity of the procedures suggested possible application in serodiagnosis. It was found that antibodies in syphilitic serum would mediate a staining reaction in smears of Treponema pallidum obtained from orchitic rabbit testes. The procedure employed was the layering method, in which intact organisms are treated on a slide with human syphilitic serum, washed, and the staining reaction is developed with fluorescein-labeled antihuman globulin.

Staining reactions were produced by syphilitic sera that reacted in the T. pallidum immobilization test. Antilipid components were likewise implicated, however, since staining was mediated by TPI-negative sera from patients with early primary syphilis and by sera from two nonsyphilitic individuals with leprosy.

3160

Thivolet, J.; Grospiron, D.; Murat, M. 1960. Demonstration, by means of immunofluorescence, of antibodies in the course of syphilitic infection. *Ann. Inst. Pasteur* 99:920-924. In French.

Experiments show that the use of immunofluorescence for the serologic diagnosis of syphilis may yield very good results. The preliminary findings obtained by the authors confirm those previously described by Borel and Durel, but it still seems impossible to propose a practical technique. The influence of many factors must be determined before any method could be adopted in clinical routine.

3170

Vaisman, A.; Hamelin, A. 1961. The use of the immunofluorescence method in the serological diagnosis of syphilis. *Prophylac. Sanit. Morale* 33:183-195.

The new method of FTA for the diagnosis of syphilis is compared on 1,176 sera and 126 spinal fluid samples with the TPI, Reiter, and Kline and Kahn flocculation tests. The FTA test was the most sensitive for detection of treponemal antibodies and was as specific as the test of Nelson. It is probable that the FTA test will become standard and will also permit differentiation of the different species of treponemes, T. pallidum, T. pertemue, and T. cuniculi.

3180

Vanini, G.C.; Mandras, A.; Ciarlini, E. 1962. Research on the possible use of indirect immunofluorescence for the detection of anti-leptospira antibodies. *Igiene Mod.* 55:11:855-865. In Italian.

The following antigens were evaluated for use in indirect FA antibody detection: live cultures; live cultures in saline suspensions, 20 per cent glucose suspensions, and 5 per cent polyvinylpyrrolidone; lyophilized cultures in each of the above three suspensions; formulated leptospira; formulated and lyophilized leptospira. Only the formulate antigen prepared by the method of Galton for macroscopic slide agglutination was satisfactory for FA. The FA test was specific but less sensitive than agglutination lysis or slide macro-agglutination.

3190

Vignali, C. 1962. The immunofluorescence test in primary syphilis. *Rass. Dermatol. Sifolograf.* 15:2:58-64. In Italian.

This is a study of serodiagnosis in primary syphilis. A rapid increase in titer was found. The speed of the test and possibility of early therapy recommend it for use.

3200

Vignali, C. 1962. Comparison of the immobilization, TPI, test with the fluorescence, FTA, test on serum of patients with various clinical conditions. G. Ital. Dermatol. 103:1:35-46. In Italian.

The TPI and FTA tests were compared on about 100 sera. FTA was positive earlier in primary syphilis. In other clinical syphilis forms the tests were equal. In latent, treated syphilis the tests agreed in 82 per cent of the cases. The FTA test was easier to perform.

3210

White, F.H.; Ristic, M. 1959. Detection of Leptospira pomona in guinea pig and bovine urine with fluorescein-labeled antibody. J. Infect. Dis. 105:118-123.

Leptospira pomona was demonstrated in the urine and kidneys of experimentally infected guinea pigs by the fluorescent antibody technique. L. pomona was demonstrated regularly by fluorescent-labeled antibody in the urine of experimentally infected calves during the shedding stage. The fluorescent antibody technique, as applied to detection of leptospires in bovine urine, proved better than darkfield microscopy. The fluorescent antibody technique, as described, possesses inherent advantages over cultural and currently used serological methods for the detection of leptospiral infection.

3220

White, F.H.; Stoliker, H.E.; Galton, M.M. 1961. Detection of leptospires in naturally infected dogs, using fluorescein-labeled antibody. Amer. J. Vet. Res. 22: 650-654.

Leptospires were isolated from the urine of 17 of 156 dogs from which satisfactory specimens were obtained by bladder tap. Sixteen of the isolates were identified as Leptospira canicola and one as L. icterohaemorrhagiae. Leptospires were detected by fluorescein-labeled antibody in the urine sediment of 14 dogs from which leptospires had also been recovered by culture. In three of these dogs leptospires were found only in the second specimen, obtained 7 to 10 days later. Leptospires were recovered by culture and were identified by silver stain and fluorescein-labeled antibody in the kidney tissue of two dogs killed 4 and 6 weeks, respectively, after first being found infected. Of 193 canine serum specimens examined by macroscopic, or Galton, antigens for leptospiral agglutinins, 46 were found positive. Leptospires were isolated from 15 of the seropositive dogs. The predominant serum agglutinins were present against L. canicola; however, three culturally negative dogs carried agglutinins only against L. icterohaemorrhagiae, and one against L. ballum only.

XIV. OTHER

3230

Biegeleisen, J.Z., Jr.; Marcus, B.B.; Nicholson, L.R.; Cherry, W.B. 1962. Fluorescent globulins for the identification of Herellea vaginicola strains in dried smears. Bacteriol. Proc. M79:82.

Herellea vaginicola has been implicated in gram-negative septicemic and meningitic infections. Each of four fluorescein isothiocyanate-labeled globulin fractions prepared from antisera to four strains of Herellea vaginicola were used to stain specifically eight strains isolated from blood of patients with septicemias. One strain was stained by group I conjugate, 5 with group II, and one each with groups III and IV conjugates. One strain was stained significantly by three of the conjugates. No other cross-reactions were observed. A group of heterologous gram-negative and gram-positive organisms were not stained with these conjugates when they were used at the diagnostic dilutions. The specificity of the reaction was confirmed by the fluorescence inhibition test and by failure to demonstrate fluorescence in smears treated with normal globulin conjugate. Results indicated that a pool of conjugates might provide a rapid, specific test for H. vaginicola in blood and spinal fluid.

3240

Cherry, W.B. 1961. The use and limitations of the fluorescent antibody technic in the identification of bacteria in body fluids and exudates and from cultures. Amer. J. Clin. Pathol. 35:256-257.

Fluorescein-labelled antibodies are potentially applicable to the detection of all bacteria. The necessity of thorough investigation of each antigen-antibody system, however, cannot be overemphasized. Each must be evaluated for rapidity, sensitivity, and specificity of staining under the environmental conditions characteristic of the disease state. Known positive controls must be included at each step. Fluorescence tests and conventional procedures should be performed simultaneously on the same specimens until it is established beyond a doubt that the former are equal or superior to the latter. The diagnostic applications of the fluorescent antibodies in bacteriology are in the early stages of development, and at the present time no fluorescence test has supplanted the corresponding conventional procedure.

3250

Cherry, W.B.; Goldman, M.; Carski, T.R.; Moody, M.D. 1960. Fluorescent antibody techniques in the diagnosis of communicable diseases. U.S. Public Health Service Publication 729. U.S. Government Printing Office, Washington, D.C. 73 p.

A history of the development of immunochemical staining procedures, with special reference to applications in the diagnostic field, has been presented. Theoretical and practical considerations of fluorescent antibody techniques were discussed. The preparation of reagents employed in immunofluorescence studies was explored and their availability was cited. The types of fluorescent antibody tests that are used most

commonly were discussed and their application to diagnostic problems in the field of microbiology was presented in some detail. Mention was made of certain practical problems that arise in the application of these tests to the diagnosis of communicable diseases.

3260

Coons, A.H. 1956. The morphological aspects of virus infections of cells as revealed by fluorescent antibody, p. 203-207. In Ciba Foundation Symp. Nature of Viruses. Little, Brown and Company, Boston.

Antibodies labelled with fluorescein have been successfully employed to detect the antigens of a number of viruses inside the infected cell: mumps, influenza, infectious canine hepatitis, vaccinia, varicella, primary atypical pneumonia, Egypt (sic), canine distemper, measles, herpes simplex, psittacosis, and in preliminary experiments, poliomyelitis. It is likely, therefore, that cells infected with most viruses against which antibody can be obtained either by the immunization of animals, or from individuals convalescent from infection, can be visualized in this way.

3270

Dashkevich, I.O.; Diakov, S.I.; Nikitin, V.M.; Osipova, I.V. 1962. Contributions to the method of processing bacteriological preparations with fluorescent antibodies. Zh. Mikrobiol. Epidemiol. Immunobiol. 33:7:101-107. In Russian.

The work deals with preparation, fixation, and staining of bacteriological preparations with fluorescent antibodies by direct and indirect methods. The investigations have demonstrated that the temperature regimen and the duration of smear staining by means of fluorescent antibodies are important factors that affect the intensity and specificity of microbial cell fluorescence. The composition of the washing liquids and the methods of fixation of the preparations had no essential effect on the capacity of bacterial cells to be stained with specific fluorescent antibodies.

3280

deRepentigny, J.; Sonea, S. 1962. Microfluorometric studies of microorganisms: III. Secondary fluorescence added by fluorescent antibodies. Ann. Inst. Pasteur 102:182-191. In French.

The specific fluorescence intensity added to bacteria by fluorescent antibodies has been determined for two bacterial species, using necessary controls. There is some non-specific staining due to heterologous fluorescent antibodies or to antibody-free coupled ovalbumin. Primary fluorescence of bacteria stained with fluorescent antibodies has been estimated. Fluorescein isothiocyanate, alone or coupled with antibodies or other proteins, shows an increase in its fluorescence when the UV irradiation is prolonged. The intensity of the bacteria stained with sera coupled with this substance may be modified accordingly.

3290

Eveland, W.C.; Smith, C.W.; Marshall, J.D.; Brown, E.R. 1959. Studies of organisms of the tribe Mimae in culture and tissue by the fluorescent antibody technique. Bacteriol. Proc. M109:91.

The tribe Mimae and serologically related Bacterium anitratum present difficulties in diagnosis because of their pleomorphism and resemblance to other gram-negative organisms. A study has been made to determine whether these organisms can be specifically identified by using fluorescent antibody. Antisera were prepared, using five different methods of antigen preparation. Direct and indirect techniques were used with sera conjugated with fluorescein isothiocyanate. Tissue sections were from laboratory-infected animals. A nonspecific serum conjugated with lissamine rhodamine RB 200 was a counterstain in the tissue sections and impression smears. Pure cultures, impression smears, and tissue sections were studied. Based on two heterologous systems, organisms could be demonstrated in both pure and mixed culture as well as in frozen and fixed tissue. Reverse screening with sera from other gram-negative species conjugated with fluorescein isothiocyanate was used against organisms being studied and indicated no serious cross-reactions to date.

3300

Hobson, P.N.; Mackay, E.S.; Mann, S.O. 1955. The use of fluorescent antibody in the identification of rumen bacteria in situ. Research 8:530-531.

Differentiation of rumen bacteria is very difficult by standard methods. Using the Coons fluorescent antibody method, it was possible to demonstrate the pleomorphism of serologically similar organisms. The English-built equipment and the problems encountered are discussed in general terms.

3310

Hobson, P.N.; Mann, S.O. 1957. Some studies on the identification of rumen bacteria with fluorescent antibodies. J. Gen. Microbiol. 16:463-471.

Fluorescent antisera to a number of isolates of rumen bacteria have been prepared and used to demonstrate the presence of these organisms in situ in rumen contents. The serological tests for a specific organism in rumen contents of a number of calves agree with the isolations of this organism from the same sources. A microscope suitable for this work, the preparation and purification of antisera, and the preparation of specimens are described.

3390

Lake, A. 1962. Tracking killer germs. Sat. Eve. Post 235:76, 78.

This article acquaints the lay public with fluorescent antibody as it is used to diagnose certain infectious diseases. Uses discussed are diagnoses of epidemic diarrhea, strep throat, rabies, venereal disease, and brucellosis. Field use in various city and county health departments is indicated, as are future diagnostic possibilities.

3330

Lipkin, M.E.; Veselov, V.A.; Pushkova, K.T. 1961. Experiences on the practical use of luminescent sera. *J. Microbiol. Epidemiol. Immunobiol.* 32:26-29.

Specificity and sensitivity of dry diagnostic luminescent sera were examined. Antisera to rabbits and to *E. coli*, *B. anthracis*, *P. tularensis*, *R. prowazeki*, and typhoid, paratyphoid, and dysentery bacilli were used. Except in the case of the two *E. coli* strains tested, 026 and 055, the method of luminescent microscopy did not give results qualifying it as a reliable means for a speedy bacteriological laboratory diagnosis. Unadsorbed anthrax antisera gave not only markedly positive results with the homogeneous strains but also results with anthracoids or pseudoanthrax bacilli. Even absorption of the anthrax antiserum with anthracoids or with a mixture of anthracoids and pseudoanthrax bacilli did not altogether abolish weakly positive reactions. Using live tularemia vaccine, it was not possible to produce a distinct luminescence with the direct or the indirect method.

3340

Meysel, M.N.; Kabanova, Ye.A.; Levina, Ye.N.; Pishchurina, M.M. 1957. Fluorescent antibodies and their application in cytology and microbiology. *Izv. Akad. Nauk SSSR, Ser. Biol.* 6:718-732. In Russian.

The fluorescent antibody method was deemed to be of great potential value to many medical fields. Techniques and their modifications for preparing reagents were investigated. Vaccinia virus and various bacteria were specifically stained. The authors felt that their technique alterations improved results of FA observations.

3350

Sinititskiy, A.A.; Diakov, S.I.; Osipova, I.V. 1959. Application of fluorescent antibodies to the detection of pathogenic microbes. *VoennoMed. Zh.* 4:35-40. In Russian.

FA is mentioned in reference to accelerated bacteriological diagnosis of infectious diseases. Various applications of the method including foreign protein tracing, homologous protein tracing, and study of pathogenic bacteria and viruses are discussed. Accelerated diagnosis and detection by means of fluorescent antibodies remains tentative, a signal method, and does not eliminate the need for carrying out classic methods of microbiological diagnosis.

3360

Sorens, S.; deRepentigny, J. 1961. Techniques for the standardization of fluorescent antibodies used in diagnostic microbiology. *Can. J. Microbiol.* 7:835-836.

Total fluorescent intensities of fluorescent sera in capillaries were measured and compared with fluorescent intensities of known quantities of fluorochromes in aqueous solutions. Changes in antibody proteins after coupling were compared by the Ouchterlony agar diffusion technique. Fluorescence of bacteria treated with fluorescent antibodies, fluorescent intensity of unstained bacteria, and the same bacteria treated with fluorescent heterologous antibodies as controls were compared.

3370

White, J.D. 1961. The use and limitations of the fluorescent antibody technic in the identification and localization of bacteria in specimens of tissue. Amer. J. Clin. Pathol. 35:257-260.

Problems and their solutions involved in the localization of bacterial antigen in tissue are discussed. Fixation methods for various types of antigens are suggested, along with the basic methods for tissue preparation; autofluorescence and nonspecific fluorescence, and methods of overcoming these problems, are included as is the importance of selecting appropriate controls.

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White, F.H.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&C		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		
1. ORIGINATING ACTIVITY (Corporate author) U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland 21701		2a. REPORT SECURITY CLASSIFICATION Unclassified
		2b. GROUP
3. REPORT TITLE IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY: I. BACTERIAL STUDIES		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (Last name, first name, initial) Sanborn, Warren R.		
6. REPORT DATE December 1965	7a. TOTAL NO. OF PAGES 143	7b. NO. OF REFS 337
8a. CONTRACT OR GRANT NO. A. PROJECT NO. None C. D.	9a. ORIGINATOR'S REPORT NUMBER(S) Miscellaneous Publication 3	
9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
10. AVAILABILITY/LIMITATION NOTICES This material is approved for domestic and foreign public release. There are no restrictions whatsoever on its distribution or on its reproduction in whole or part.		
11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland 21701	
13. ABSTRACT This volume is one of a series of six annotated bibliographies on various aspects of immunofluorescence and its use. Citations cover the period 1905 through 1962. Volume I contains 337 annotated literature citations, arranged according to major subject areas, and a complete author index.		

DD FORM 1473
1 JAN 64

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Security Classification